DESIGN AND EVALUATION OF A PILOT-SCALE ELECTRO-BIOCHEMICAL REACTOR

by

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STATEMENT OF THESIS APPROVAL

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ABSTRACT

The removal of nitrate, arsenic, and selenium from mining influenced waters (MIW) to ultra-low levels remains difficult. The results achieved from the design and evaluation of a novel Electro-Biochemical Reactor represent a breakthrough in the removal of metals and inorganics, including nitrate, arsenic, and selenium, from mine water.

The Electro-Biochemical Reactor (EBR) can be employed in multiple configurations; its simplest form is a single pass, fixed-bed, up-flow bioreactor for the removal of metals, metalloids, and inorganics from water. The Electro-Biochemical Reactor relies on direct provision of electrons into the bioreactor to develop electron donor/acceptor environments for the transformation of contaminants. The application of an applied voltage (1-3 VDC) potential provides readily available electrons for enhancement of microbial donor/acceptor metabolic reactions and helps control the oxidation/reduction potential of the bioreactor environment.

Several configurations of the Electro-Biochemical Reactor (EBR) were tested at bench-scale (1 Liter/day) and pilot-scale (1-4 Liters/minute) using mine effluent waters from two different mine sites to remove nitrate, arsenic, and/or selenium to effluent target levels.

Results indicate selection and screening of different microbes and microbial support material (MSM) for site-specific waters affected contaminant removal performance; selection of the proper microbes and MSM resulted in significantly improved contaminant transformation kinetics. Side by side bench-scale tests of an EBR vs. CBR (conventional bioreactor) on mine water containing nitrate and arsenic were conducted. In these tests, EBR performance was 13% better than the conventional bioreactor, the CBR, removing arsenic from 350 ppb to a final concentration of 12 ppb versus a final concentration of 50 ppb for the CBR. Pilot-scale tests of the EBR system were conducted at a closed heap-leach Gold mine for nitrate and arsenic removal and at a Base Metal mine for selenium removal from site waters. Pilot-scale results from the gold

mine waters indicate the EBR system was able to remove arsenic from 800 ppb to an average of 50 ppb and 20 ppm of nitrate to nondetectable levels in less than 10 hours. Further EBR pilot-scale tests were conducted at a Base Metal mine for selenium removal. The EBR system successfully removed selenium from an average of 2.73 ppm to less than 0.002 ppm in approximately 8 hours.

Additionally, the EBR system's ability to remove other metals and inorganics is shown in Table 22.

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LIST OF ACRONYMS

WHO	World Health Organization
As	arsenic
Se	selenium
EBR	Electro-Biochemical Reactor
MIW	mining influenced water
HRT	hydraulic retention time
ANFO	ammonium nitrate and fuel oil
REDOX	reduction oxidation
K_d	partition coefficient
U.S. EPA	United States Environmental Protection Agency
CWA	clean water act
O&M	operation and maintenance
MFC	microbial fuel cell
DC	direct current
ATP	adenosine triphosphate
MV	methyl viologen
TDS	total dissolved solids
TOC	total organic compound
TSS	total suspended solids
BOD	biological oxygen demand
COD	chemical oxygen demand
WAD	weak acid dissociable
ICP-MS	inductively coupled plasma – mass spectrometry

DO	dissolved oxygen
TSA	tryptic soy agar
TSB	Trypticase™ Soy Broth
OD	optical density
PES	Polyethersulfone
EQ	equalization
PVC	polyvinyl chloride
GPM	gallon per minute
LPM	liters per minute
MSM	microbial support material
GAC	granular activated carbon
CBR	conventional bioreactor
C	Celsius
ppm	parts per million
ppb	parts per billion
COC	contaminants of concern

INTRODUCTION

Background

The impact from anthropogenic activities is now the most important factor affecting world water quality and the world's largest health risk is polluted water. It is estimated, by the World Health Organization (WHO), that 1.1 billion people do not have access to clean drinking water (1). The primary water-quality issues caused by human activities include organic material, heavy metals, acidic atmospheric deposition and runoff, salinization, nutrients (primarily nitrogen and phosphorus), pathogenic agents including bacterial pathogens, enteric viruses, and protozoan's, suspended sediments, oil and grease, synthetic organic compounds, thermal pollution, exotic and invasive species, pesticides and herbicides, and radioactivity (2). Anthropogenic activities that contribute to the degradation in water quality include but are not limited to urban and industrial development, farming, mining, combustion of fossil fuels, stream-channel alteration, animal feeding operations, and electrical generation (3).

Metals, metalloids, and inorganics are essential for living organisms and occur in all natural ecosystems; however, their presence at elevated concentrations within the water system can impact quality of life, health, and the environment. Anthropogenic activities disturb the natural environment and can cause the release of metals, metalloids, and inorganics into the environment. For example, mining disturbs stabilized mineral deposits rich in metals, increases erosion, and mobilizes metals, such as selenium and arsenic, into streams and waterways. Additionally, inorganic compounds are introduced into the environment via chemicals we use in mining. For example, such compounds include but are not limited to ammonium nitrate (blasting) and lime (pH adjustment and precipitation).

This thesis represents an overview of the work completed in the development of the Electro-Biochemical Reactor (EBR) from bench-scale (1 l/d) to pilot-scale (1 l/m) for the removal

of nitrate, arsenic, and selenium at two different mine sites. The removal of contaminants from mining influenced waters (MIW) continues to be costly, difficult, and represents a significant operational issue in the development of any mine. The EBR represents a breakthrough for MIW treatment with respect to economics and reliability. The principle applied to the EBR system is directly providing electrons to microbes through an applied potential delivered through a set of electrodes. One amp of electricity contains 6.241×10^{15} electrons per second (4). This applied potential to the EBR system and the corresponding current results in a reduced need for nutrients and increased microbial kinetics for reduction oxidation processes (5) (6).

Research Objectives

This research is focused on elucidating basic concepts surrounding optimizing the function and operation of the electro-biochemical bioreactor. The basic concepts stated in the research objectives are part of an overall project to develop the EBR system into an effective wastewater treatment process for the removal of metals and inorganics from mining wastewaters. The research and testing conducted is focused on nitrate, arsenic and selenium. Bench-scale testing was used to research and validate concepts that could then be developed and tested at pilot-scale. Bench-scale research and testing is necessary, but once the research concepts are validated, pilot-scale testing to further validate the basic concepts developed at bench-scale, is required to completely validate the EBR process concepts using on-site tests to examine the effects of changing water quality and other environmental and system parameters on EBR contaminant removal performance.

The research and testing objectives to achieve this goal are:

- Objective 1. Determine if contaminant transformation kinetics can be enhanced through selection of microorganisms. This will be achieved by developing and conducting screening tests for evaluation of microorganisms that exhibit the best contaminant transformation capabilities for nitrate, arsenic, and selenium from actual contaminated mining waters.

  - Compare growth and contaminant removal will be examined using different microbial support materials (MSM) – pumice and carbon with different functional groups and pore sizes.

- Objective 2. Design and conduct bench-scale EBRs and test protocols to examine and validate the EBR process using microbes and MSM to determine their function in an EBR environment using various electrode placements to introduce electrons into the bioreactor for optimization of microbial function and oxidation-reduction potential (ORP) control. In accomplishment of these objectives, determine the process steps required to treat a specific mine water and define optimal electrode placement, nutrient requirements, and hydraulic retention times (HRT) necessary to achieve discharge goals for arsenic, nitrate, and/or selenium containing mining waters.
- Objective 3. Demonstrate that research results obtained in bench-scale experiments using concepts validated in Objectives 1 and 2 are transferrable to both bench- and pilot-scale EBR systems.
- Objective 4. Demonstrate that research results obtained in bench-scale experiments using direct electron addition, through various electrode placements, are validated in both bench- and pilot-scale EBR systems.

Validation of research concepts will be achieved through two different bench-scale tests and two different pilot-scale tests. Each bench and pilot test builds on design and evaluation of scale-up concepts developed with a goal to conduct successful on-site pilot-scale EBR systems to meet discharge goals. For example, accomplishment of each scale-up objective will be completed in a staged manner; first a pilot system will be designed and tested for initial concept validations at a mine site to remove nitrate and arsenic. Results from this test will be evaluated and adjusted to further optimize the EBR concepts. Next, a second pilot system based on concept refinements noted will be developed and tested at a second mine site. This test will be focused on selenium and general metal and inorganics removal for a more finalized assessment of the EBR concepts, to validate scale-up concepts and assess their potential for treatment of mining wastewaters.

Thesis Organization

The first section provides the background of the problem and defines the objectives for this thesis.

The second section offers a literature review of the impact of mining and industrial activity on nitrate, arsenic, and selenium discharges into the environment. The discussion includes a review of nitrate, arsenic, and selenium properties, occurrences, and health hazards.

Discussions of reduction/oxidation potential with corresponding Eh-pH diagrams for nitrate, arsenic, and selenium in O_2-H_2O systems as well as corresponding speciation graphs are included. A graph of standard electrode potentials for nitrate, arsenic, and selenium is included in this section as well.

Further topics reviewed include current treatment methods for nitrate, arsenic, and selenium as well as costs, advantages, and disadvantages. An analysis of energy sources and requirements needed to reduce nitrate, arsenic, and selenium are examined along with biomediated removal of nitrate, arsenic, and selenium in the context of the EBR system. The current state and future trends of sources impacting nitrate, arsenic, and selenium release are examined.

The third section reveals the design, materials, and methods used to construct the bench-scale and pilot-scale EBR systems. Flow, Hydraulic Retention Time (HRT), pH, temperature, and target contaminant concentrations as well as all operational procedures are discussed with reference to the EBR design in this chapter in keeping with the main focus of this thesis. Site specific operational and water chemistry parameters are given.

The results section provides data from two different bench-scale tests and three different pilot tests at two different mine sites, one for nitrate and arsenic, and one for selenium and includes a discussion of the results presented. Two of the pilot tests results represent a year-over-year test and are used as a comparison of modifications to the pilot-scale EBR system.

The fifth section provides the overall thesis conclusions and an analysis of contaminate removal are presented. The appendix includes graphs of all data collected during bench- and pilot-scale concept validation testing.

LITERATURE REVIEW

Mining's Impact on Nitrate, Arsenic, and Selenium in Water

Ore is defined as "aggregate of economically important minerals that are sufficiently rich to separate for a profit" (7). Ore grade is defined as "The concentration of valuable mineral contained within an ore" (7). The ore grade will have a direct impact on the amount of ore needed to be processed to achieve a certain quantity of valuable mineral. Although more than 3,500 mineral species are known, only about 100 are considered ore minerals. The term originally applied only to metallic minerals but now includes such nonmetallic substances as sulfur, calcium fluoride (fluorite), and barium sulfate (barite). Ore is always mixed with unwanted rocks and minerals, known collectively as gangue. The ore and the gangue are mined together and then separated. The desired element is then extracted from the ore. The metal may be still further refined (purified) or alloyed with other metals (8). Metal ores are generally oxides, sulfides, and silicates with native metals, such as copper, and noble metals, such as gold, occurring much less frequently. Arsenic and selenium are mainly found in sulfide deposits of gold, copper, iron, lead, and zinc. Arsenic occurs primarily in pyrite, arsenopyrite, and as arsenides and sulfosalts. Selenium is most common in chalcopyrite, bornite, and pyrite (9) (10).

Mining requires the processing of large volumes of ore (rock containing valuable minerals). Typically, ore contains very little valuable minerals; as an example, the average global copper ore grade is 0.8% (11); as a result, 99.2% of the ore, also referred to as gangue or tailings, is discarded into large heaps or returned to the underground mine and used as backfill. During mineral processing, the ore is typically mined, crushed, and ground (comminution) to a fine powder to "liberate" the valuable minerals for further separation. This process of liberation not only frees the valuable mineral but all other components of the ore as well, which includes nitrate from blasting and natural components of selenium and arsenic.

Mining and mineral processing can introduce large quantities of nitrates, arsenic, and selenium into water. Nitrogen is typically introduced into mine influenced water through the use of explosives. The majority of explosives are ammonium nitrate based and accounted for 98% of all U.S. industrial explosives sales (12). 92% of all explosives are used in the mining industry (13). A recent study found that 1 tonne (5.2% of total monthly use) of ammonium nitrate and fuel oil (ANFO) was entering the mine water collection system each month at a particular mine site in Canada (14). ANFO enters the mine water collection system in several ways, both through spillage or poor handling techniques and from incomplete detonation (15).

It has been estimated that 35 x 10^6 kg yr^{-1} of arsenic is discharged into the environment every year from anthropogenic activities (16) and naturally occurring arsenic pollution affects nearly 140 million people in 70 countries on all continents (17).

Total selenium discharge to the environment is estimated at 12 x 10^6 kg yr^{-1} and about 43% comes from anthropogenic activities. Of that 43%, 40% is considered to be from mining, smelting and industrial processes and the remaining 60% from the combustion of fossil fuels (18).

Characteristics of Nitrate, Arsenic, and Selenium in the Environment

Nitrate (NO_3^-) is a polyatomic ion and nitrates are the salts of nitric acid (HNO_3). Nitrate is mainly used as an explosive in the form of ammonium nitrate/fuel oil. ANFO is 94% ammonium nitrate (NH_4NO_3) and 6% number 2 fuel oil. 90% of explosives are used in coal mining, quarrying, metal mining, and civil construction (19). ANFO accounts for an estimated 80% or 4,400,000,000 pounds of all explosives used in the United States in one year (20).

Arsenic (As) is a group V element on the periodic table and is classified as a metalloid. Arsenic has an estimated abundance within the earth's crust at an average concentration of 1.8 mg/kg (21). Arsenic has four oxidation states (-3,0,+3, and +5) and in ground/surface waters, the +3 and +5 oxidation states are most prevalent as oxyanions arsenite (As_2O_3 at pH □ 9-11) and arsenate (AsO_4^{3-} at pH □ 4-10) (22). Arsenic is somewhat unique among the heavy metalloids and oxyanion-forming elements (As, Se, Sb, Mo, V, Cr, U, Re) in its ability to dissolve in water at all pH values and its mobility under a wide range of redox conditions. Two important factors

affecting arsenic speciation are pH and redox potential (Eh). At pH values below 7 under oxidizing conditions, $H_2AsO_4^-$ is the dominate species; while above 7, $HAsO_4^{2-}$ dominates. Under reducing conditions at pH values below approximately 9.2, H_3AsO_3 is predominant (23).

Selenium (Se) is a group VI element on the periodic table and is classified as a non-metal. Selenium is estimated to have a crustal abundance of 0.05 mg/kg (24). Selenium has four oxidation states (-2, 0, +4, +6) with the +4 and +6 oxidation states of selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) being the most prevalent in ground/surface waters (25). Selenium is mobile as the selenate oxyanion (SeO_4^{2-}) under oxidizing conditions but is immobilized under reducing conditions possibly due to absorption of its reduced form, selenite (SeO_3^{2-}), or through reduction to its elemental form (Se^0) (22) (26) (27).

The presence of arsenic and selenium in the environment at elevated levels is cause for concern as they can accumulate through the food chain and cause reproductive disorders. The chemical characteristics of Se and As are dominated by the fact that Se and As readily change oxidation state or chemical form through chemical or biological reactions that are common in the environment. Therefore, rather than solubility equilibrium controlling As and Se mobility, it is usually controlled by redox conditions, pH, biological activity, and adsorption/desorption reactions. Arsenic and selenium enter the aquatic environment as a result of both natural and anthropogenic sources, including: natural weathering, agricultural drainage, combustion of fossil fuels, and various mining and mineral processing activities. Mining can accelerate the release of As and Se into the environment by exposing waste rock to air and water (28).

Health Effects from Nitrate, Arsenic, and Selenium

Some metals and inorganics are essential for human health in trace amounts. They are essential because they form an integral part of one or more enzymes involved in a metabolic or biochemical process (29) (30). However, all metals and nonmetals are toxic at a certain level (31) (32).

Nitrate Health Effects

Nitrate mainly enters the body through food; however, in one particular study, it was found to have entered through drinking water (NO_3>20 mg/L) used to prepare an infant's baby formula (33). Nitrate toxicity is generally acute to subacute with symptoms seen from a few hours to a few days after consumption of large amounts of nitrate, 1.8 to 3.2 mg/kg/day. The health effects of nitrates in humans include methemoglobinemia or blue baby syndrome, headache, dizziness, weakness, and difficulty breathing (34). Health effects in animals include abdominal pain, diarrhea, muscular weakness, and poor condition (35).

Arsenic Health Effects

Arsenic can enter the human body through skin absorption, ingestion, or inhalation. Arsenicosis is toxicity due to high levels of arsenic in the body. Arsenic poisoning can cause major health complications, including death. Once arsenic has entered the body, it is distributed to a large number of organs, including the lungs, liver, kidney, and skin (36) (37). Acute intoxication usually occurs within 30 minutes and symptoms include a metallic or garlicky taste and difficulty swallowing. Other symptoms may include muscular pain, weakness, gastrointestinal symptoms, and flushing skin (38). Small amounts of arsenic (<5 mg) can result in diarrhea and vomiting but usually subside after 12 hours and treatment is reported to not be necessary. The lethal dose of arsenic in acute poisoning ranges from 100 mg to 300 mg (39). The estimated acute lethal dose of inorganic arsenic is about 0.6 mg/kg/day (40).

Selenium Health Effects

Selenium can enter the human body through food, water, and air; however, the typical pathway is through food. High blood levels of selenium (>100 mcg/d) can result in toxicity, which is a condition called selenosis (41). Symptoms of selenosis include gastrointestinal upsets such as nausea, vomiting, abdominal pain and diarrhea, hair loss, white blotchy nails, garlic odor breath, fatigue, irritability, and mild nerve damage hyperreflexia. Extreme cases of selenosis can result in cirrhosis of the liver, pulmonary edema, thrombocytopenia, thyroid problems, and death

(42). Chronic toxicity of selenium is the result of long-term ingestion, usually from weeks to months, and is believed to be caused by daily intake concentrations greater than 0.015 mg/kg (43). High blood levels of selenium (>100 mcg/d) can result in toxicity which is a condition called selenosis (41).

Bioconcentration and Biomagnification of Nitrate, Arsenic, and Selenium

The underlying reason for the removal of nitrate, arsenic, and selenium from water to such low levels is based on the concepts of biological half-life, bioconcentration, and biomagnification. The biological half-life refers to the time it takes for a substance to lose half of its physiological activity. Bioconcentration refers specifically to the uptake and accumulation of a pollutant from water to the first organism in the food chain at a rate greater than at which the substance is lost. Biomagnification is an increase in concentration of a pollutant from one link in a food chain to another (44) (45). We are concerned about these phenomena because together they mean that even small concentrations of nitrate, arsenic, and selenium in the water can find their way into organisms in high enough concentrations to cause adverse health effects. The four criteria used to determine the potential for adverse health effects include contaminate lifespan, mobility, solubility in fat, and biological activity. Contaminant lifespan, fat solubility, and biological activity vary greatly from organism to organism and in the case of selenium and arsenic between different oxidation states (46) (47). However, contaminant oxidation state and in turn mobility is greatly influenced by the geochemical conditions Eh-pH.

Redox Potential and pH Effects on Nitrate, Arsenic, and Selenium Speciation in MIW

Redox conditions and pH exercise important controls in water chemistry. The influence of redox potential and pH on nitrate, selenium, and arsenic speciation is shown in the Eh-pH diagrams, Figures 1, 2, and 3. Figure 4 provides a legend for the Eh-pH diagrams. Redox potentials in natural waters range from about -400 mV to +800 mV at pH 7 to 8 (48).

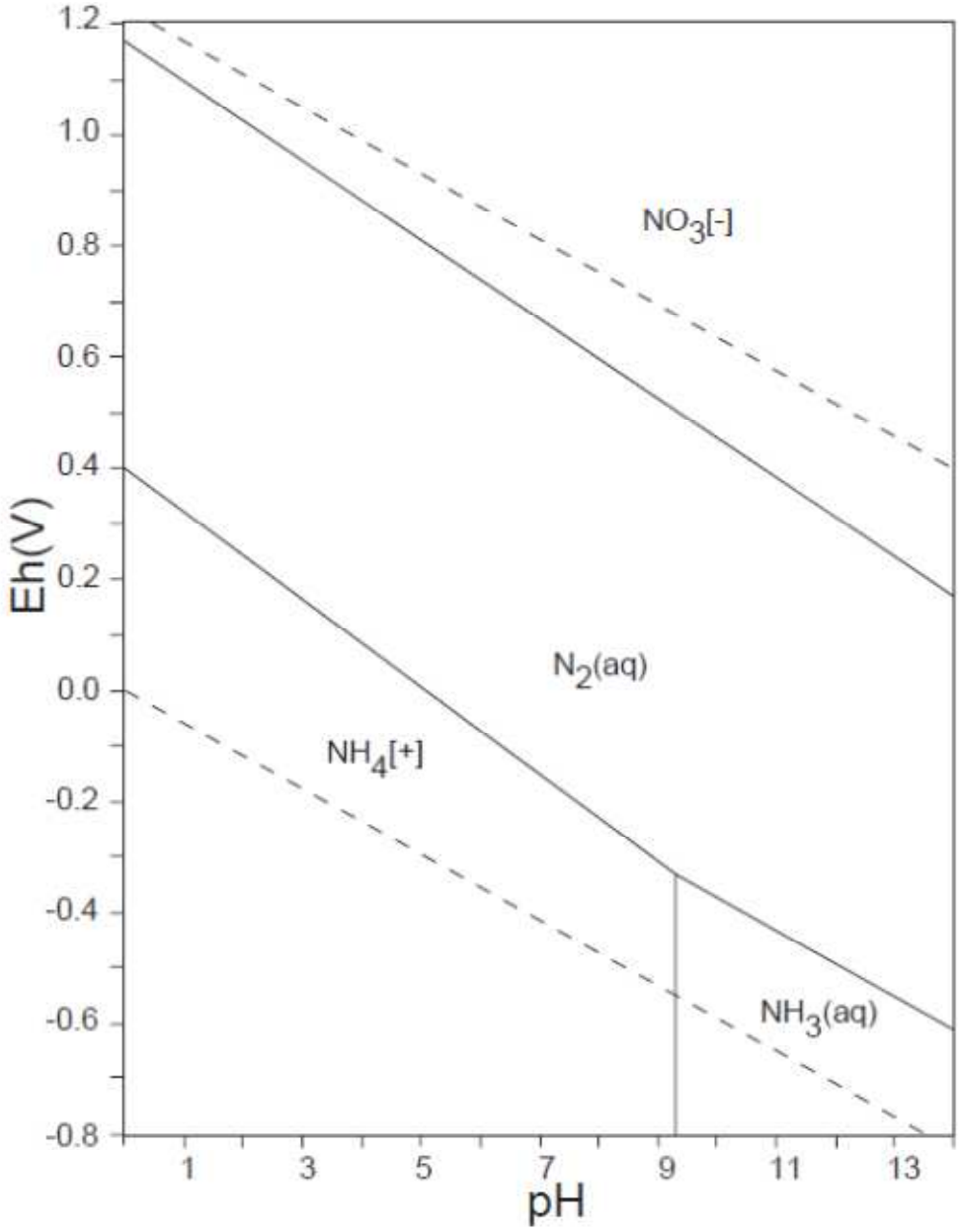

Figure 1. Eh-pH diagram for nitrogen (N) species in the system N-O-N at 25^0 C and 1 bar total pressure (26).

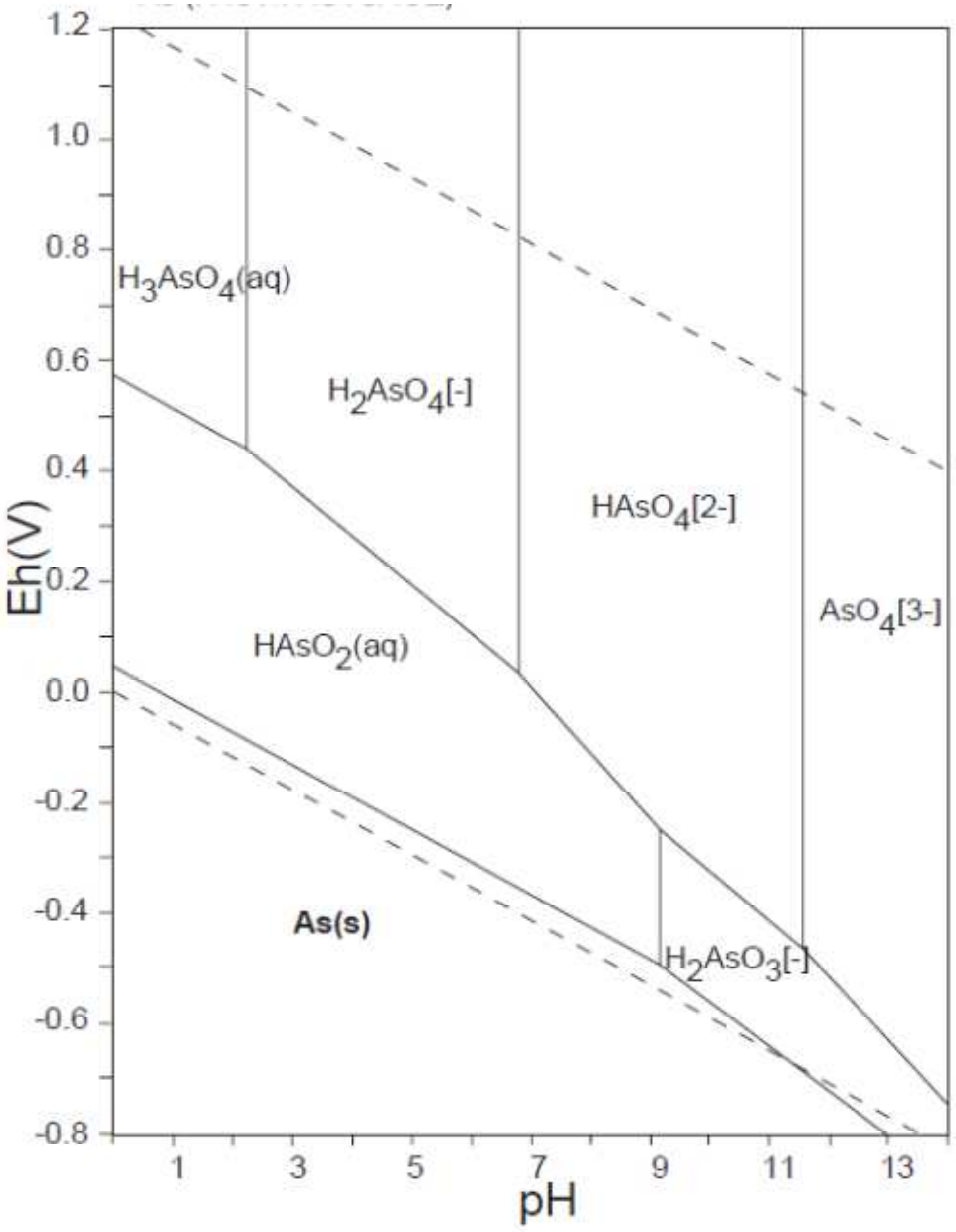

Figure 2. Eh-pH diagram for As species in the system As-O-H at 25^0 C and 1 bar total pressure (26).

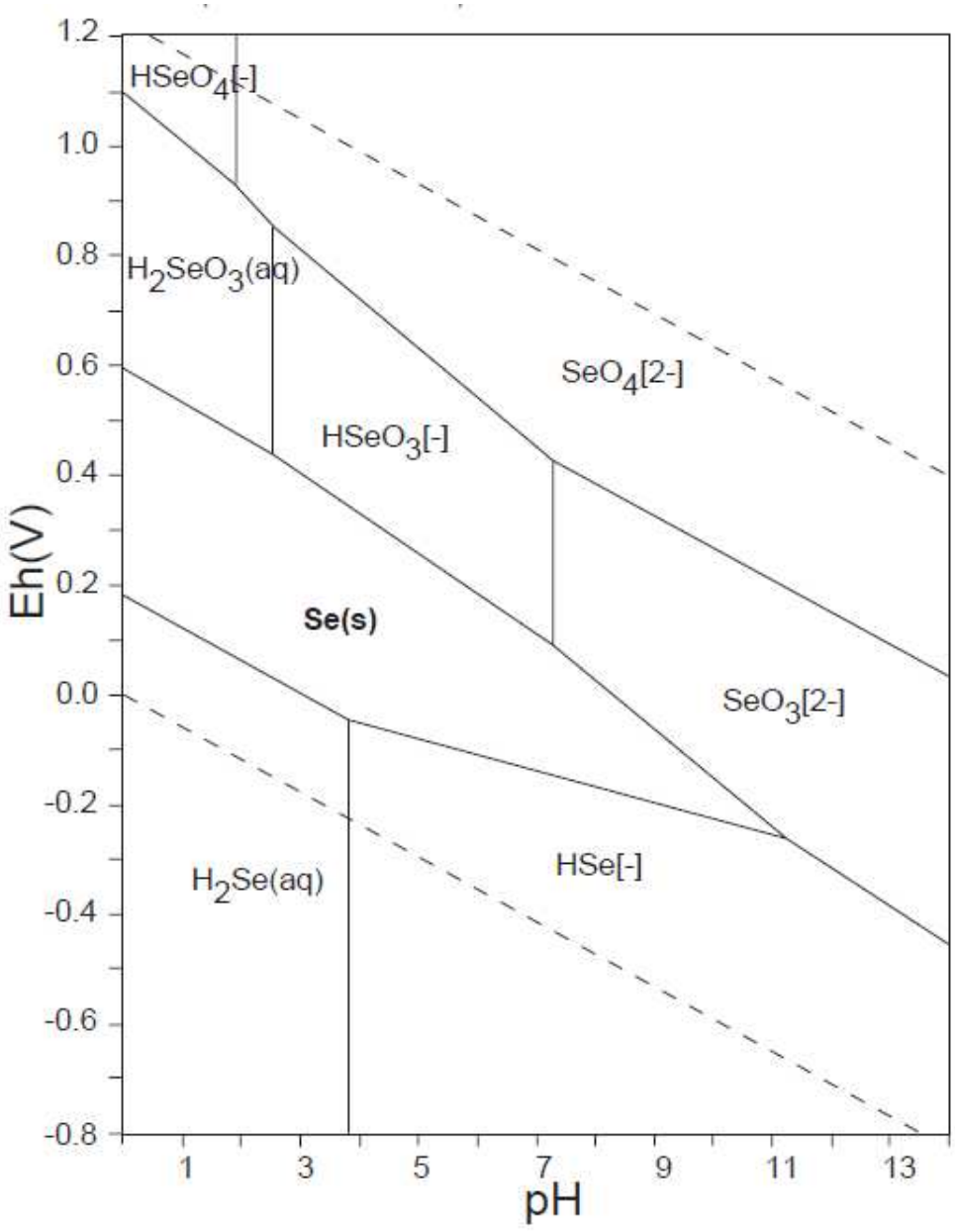

Figure 3. Eh-pH diagram for Se species in the system Se-O-H at 25^0 C and 1 bar total pressure (26).

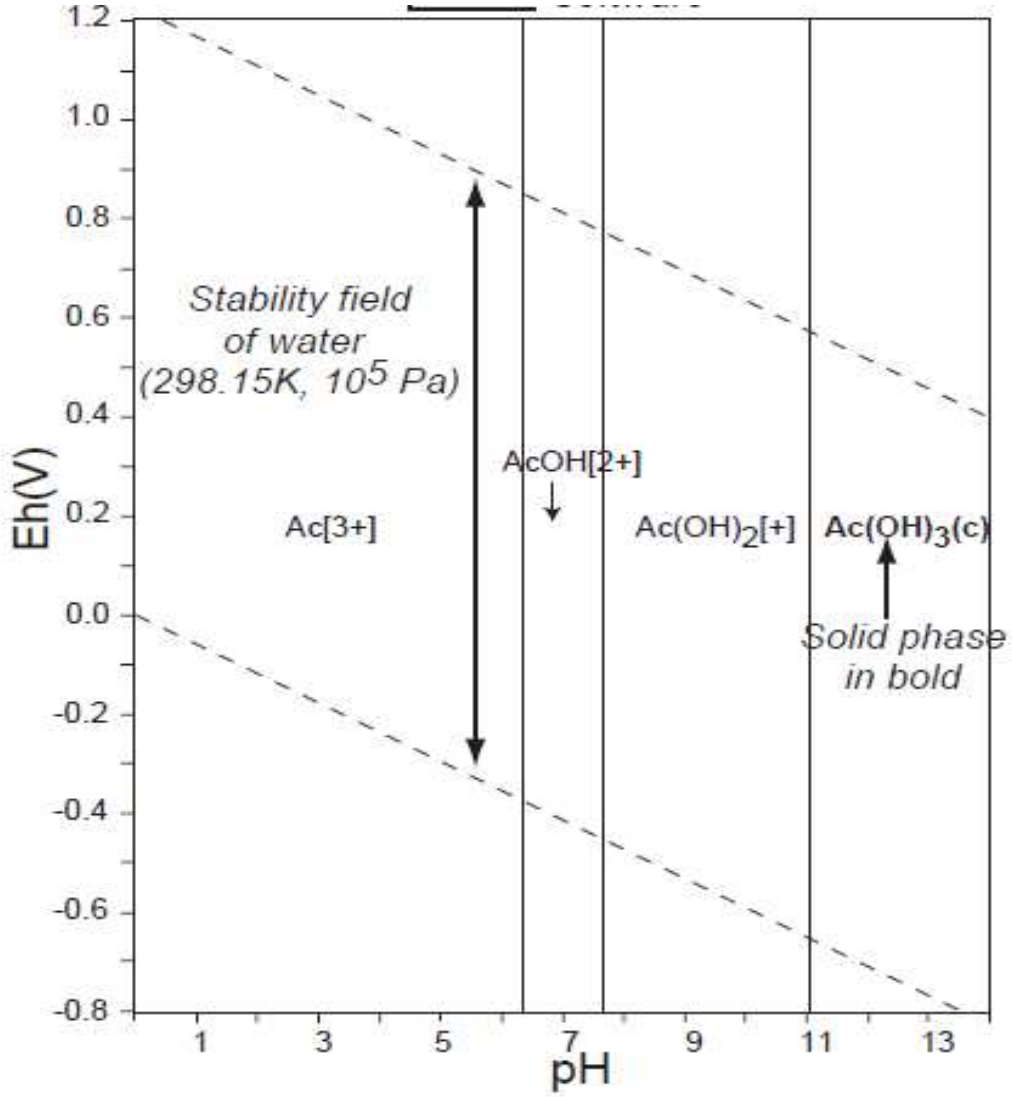

Figure 4. Legend Eh-pH diagram (26).

Based on Eh-pH diagrams, in fresh water environments, nitrogen is most commonly found as nitrate, arsenic as arsenite and arsenate, and selenium as selenite and selenate. Water treatment methods based on reduction of these species tend to provide reductive environments, whereas nitrate is reduced to nitrogen gas and arsenic and selenium oxyanions are reduced to elemental metals.

Eh-pH diagrams provide information on the chemical species and the oxidation state of nitrogen, arsenic, and selenium in water. Speciation and oxidation states of the particular contaminant will directly affect its biotoxicity and mobility in water. Biotoxicity was elucidated upon

in the health effects section of this thesis; however, speciation directly affects contaminant mobility. Contaminant mobility is evaluated by the Partition coefficient or Sorption Distribution coefficient (49). The Partition coefficient is the ratio of sorbed contaminant to the total dissolved concentration. As water with contaminants travels through soil and surface water systems, contaminants will undergo sorption to the solid matrix and a reduction in contaminant concentration will occur and this will affect overall contaminant transport. The equation used to calculate the Partition coefficient is:

$$K_d = \frac{\text{sorbed contaminant concentration (mg/kg)}}{\text{Dissolved contaminant concentration (mg/L)}}$$

For a particular contaminant, K_d values are dependent upon various geochemical characteristics of the soil and porewater. The parameters that have the greatest influence on the magnitude of K_d are pH, ORP, and the nature and concentration of sorbents associated with the soil or surface water. The partition coefficient measures how hydrophilic or hydrophobic a particular contaminate is. Table 1 illustrates average ranges of nitrate, arsenic, and selenium. The lower the K_d value is, the more mobile the particular contaminant species will be.

Bacterial Nutrition

All organisms must find in their environment all of the substances required for energy generation and cellular biosynthesis. The chemicals and elements of the environment that are utilized for bacterial growth are referred to as nutrients or nutritional requirements. The major nutritional requirements of a bacterium are revealed by the cell's elemental composition. The major elements consist of C, H, O, N, S, P, K, Mg, Fe, Ca, Mn, and traces of Zn, Co, Cu, and Mo. The general physiological functions of the elements are outlined in Table 2.

Table 2 does not include trace elements. Trace elements are metal ions required by certain cells in such small amounts that it is difficult to detect them. As metal ions, trace elements usually act as cofactors for essential enzymatic reactions in the cell. The usual cations that qualify as trace elements in bacterial nutrition are Mn, Co, Zn, Cu, Cr, Se, Fl, I, and Mo.

Table 1. Average K_d values for nitrate and the most common oxidation states of selenium and arsenic.

Contaminant	Average K_d Value	Source
Nitrate	0.001 – 0.7	(50)
Selenite	15.48 – 20.42	(51)
Selenate	8.02 – 8.64	(51)
Arsenite	1 – 8.3	(52) (53)
Arsenate	1.9 - 18	(52) (53)

Table 2. Major elements; their sources and functions in microbial cells.

Element	% of dry weight	Source	Function
Carbon	50	Organic compounds, $C0_2$	Main constituent of cellular material
Oxygen	20	H_2O, organic compounds, CO_2, O_2	Constituent of cell material and cell water
Nitrogen	14	NH_3, NO_3, organic compounds, N_2	Constituent of amino acids, nucleic acids nucleotides, and coenzymes
Hydrogen	8	H_2O, organic compounds, H_2	Main constituent of organic compounds and cell water
Phosphorus	3	Inorganic phosphates (PO_4)	Constituent of nucleic acids, nucleotides, phospholipids, lipopolysaccharide, and teichoic acids
Sulfur	1	SO_4, H_2S, S^0, organic sulfur compounds	Constituent of cysteine, methionine, glutathione, and several coenzymes
Potassium	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium	0.5	Magnesium salts	Inorganic cellular cation and cofactor for certain enzymatic reactions
Calcium	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores
Iron	0.2	Iron salts	Component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions

Current Treatment Methods for the Removal of Nitrate, Arsenic, and Selenium

Water is ubiquitous with mining and in addition to large quantities of gangue, mining produces large quantities of wastewater with elevated levels of nitrate, arsenic, and selenium. Nonferrous mining wastewater can be generated in three ways and includes process water, mine drainage, and site stormwater release. Table 3 provides a definition of different mine water sources for classification and the U.S. federal regulatory code under which the discharge is governed.

Although mining water discharge is not regulated by drinking water standards, effluent discharge concentrations guidelines can be quite strict. Mine water discharge can impact water quality used for drinking water, fisheries, and other animal and waterfowl life exposed to these impacted waters. Discharge concentrations guidelines produced by the U.S. EPA (Environmental Protection Agency) for protection of aquatic life and human health for surface waters is published pursuant to section 304(a) of the clean water act (CWA). This document establishes

Table 3. U.S. EPA's hardrock mining framework.

Waste Stream	**Definition**
Process wastewater	"...any water which, during manufacturing or processing, comes into direct contact with or results from the production or use of any raw material, intermediate product, finished product, byproduct, or waste product." (40 CFR 122.22)
Mine drainage	Mine drainage includes water drainage from refuse, storage piles, wastes, rock dumps, and mill tailings derived from the mining, cleaning, or concentration of metal ores. Mine drainage may include process water still contained in the mine. Stormwater runoff and infiltration can contribute to mine drainage. "...any water drained, pumped, or siphoned from a mine." (40 CFR 440.132)
Industrial stormwater	Stormwater means rain water runoff, snow melt runoff, surface runoff, and surface drainage from mining and mineral processing activities. Industrial facilities are required to obtain permit coverage for stormwater if they have a point source stormwater discharge associated with an industrial or commercial activity from their property either directly to waters of the United States or to a municipal separate storm sewer system. "... the discharge from any conveyance which is used for collecting and conveying storm water and which is directly related to manufacturing, processing or raw materials storage areas at an industrial plant. ... (40 CFR 122.26)

Source: Adapted from EPA and Hardrock Mining: A Sourcebook for Industry in the Northwest and Alaska (EPA, 2003).

recommended water quality criteria for approximately 150 contaminates, including nitrate, arsenic, and selenium. The maximum effluent level for nitrate-N is 10 mg/L, the maximum effluent of selenium and arsenic for aquatic life are 150 µg/L and 5 µg/L, in fresh water. Recommended maximum concentration levels of selenium and arsenic in drinking water for humans are 0.018 µg/L and 5 µg/L, respectively.

The removal of arsenic, selenium, and nitrogen compounds from MIW for discharge into the environment has proven to be costly. Current technologies used to remove these compounds from mining process/wastewater fall into three broad categories; physical, chemical, and biological. Examples of each and cost range for treatment are presented in Table 4. There is no panacea for the treatment of MIW, or any other wastewater for that matter, and each treatment technology has advantages and disadvantages. The evaluation of any technology must be carefully considered based on operational issues associated with that technology and water chemistry. Table 5 discusses the operational issues associated with physical, chemical, and biological treatment of nitrate, arsenic, and selenium associated with the treatment of MIW.

Almost all metal and inorganics removal technologies, excluding physical methods, are based on redox reactions, which are driven by the Eh/pH regime and are often limited by electron availability. Table 6 provides examples of the half reactions and electrons required to reduce nitrate, arsenic, and selenium into nitrogen gas and elemental arsenic and selenium. For example, it takes 6 electrons to reduce 1 molecule of selenate to elemental selenium, 10 electrons to reduce 2 nitrate (NO_3) molecules to N_2, and 5 electrons to reduce 1 molecule of arsenate to elemental arsenic. A further issue surrounding MIW and the necessity for electrons is the presence of co-contaminants. Nitrate, commonly present in MIW, due to the use of ANFO, is a preferred electron acceptor over arsenic and selenium ions (Table 6); electron acceptors are reduced in the process. MIW rarely contains only nitrate, arsenic, and selenium and in a survey of 71 mines, 74% identified metals, anions and nitrogen compounds, metalloids and oxyanions as present in elevated levels in mine site water (54).

Table 4. Metals and inorganics removal categories and costs.

Treatment Type	Treatment cost range per 1000 U.S. gallons	References
Physical (reverse osmosis, nanofiltration, ion exchange, and evaporation)	$5.00-$20.00	(55) (56) (57) (58)
Chemical (iron reduction, precipitation, cementation, electrocoagulation, photoreduction)	$0.50-$9.00	(59) (57) (58)
Biological (algal volatilization, active, passive and in situ microbial reduction, wetlands)	$0.20-$1.50	(60) (57) (58)

Table 5. Advantages/Disadvantages of physical, chemical, and biological treatment technologies.

Treatment technology	Advantage	Disadvantage	References
Physical	Reliable, well understood, scalable, able to meet regulatory compliance	Membrane fouling, may require pretreatment, brine disposal, high operating pressures, resin regeneration, sulfate competition, inability to remove selenate and arsenate	(61) (57) (58) (62)
Chemical	Proven, effective technology for removal, EPA best available technology demonstrated	High chemical consumption, waste disposal, most effective with selenite and arsenite	(63) (64) (65) (66)
Biological (Active)	Proven technology, able to meet regulatory compliance, small footprint, low operating cost compared to physical and chemical	High capital costs, pretreatment may be necessary, plugging by selenium precipitates, re-oxidation and mobilization of Se possible	(64) (67)
Biological (passive/in-situ)	Low capital and O&M costs, little to no active supervision, ability to treat high flow rates, able to meet regulatory limits	Long-term performance not well understood, large footprint, generation of anoxic conditions toxic to wildlife	(68) (64)

Table 6. Standard electrode potentials in aqueous solutions at 25^0 C and 1 atm (69)**.**

Compound	**Half-Reaction**	**Half-Cell Potential @ 25 ^{0}C**
Nitrate	$NO_3^-(aq) + 6H^+(aq) + 5e^- \rightarrow 1/2N(g) + 3H_2O(l)$	0.96 V
Selenate	$SeO_4^{2-} + 2H^+ + 2e^- \rightarrow SeO_3^{2-} + H_2O$	0.880 V
Selenite	$SeO_3^{2-} + 6H^+ + 4e^- \rightarrow Se(s) + 3H_2O$	0.875 V
Arsenate	$AsO_4^{3-} + 8H^+ + 5e^- \rightarrow As(s) + 4H_2O$	0.648 V
Arsenite	$As_2O_3(s) + 6H^+ + 6e^- \rightarrow 2As(s) + 3H_2O$	0.234 V

In chemical precipitation methods, electron donor in a form of a chemical compound must be provided, such as Na_2S. In conventional biological treatment systems, organic carbon-based nutrients act as electron donors and is shown in Table 7 and electron donors are oxidized in the process. The carbon-based electron donors must be supplied in excess to provide high electron availability, for reduction of contaminants of interest, as well as co-contaminants which translates to higher operational expenses associated with the treatment. Glucose in the form of molasses provides only 24 electrons upon complete oxidation to CO_2 and H_2O. That means that in theory, about half a mole of glucose is required to reduce one mole of selenate and one mole of nitrate to their elemental form, corresponding to 90 grams of glucose. However, the oxidation of glucose through glycolysis requires a biological kinetic component for metabolism. The anaerobic glycolysis system provides energy from about 10 seconds to 2 minutes and provides about 5% of glucose's energy potential (70) (71). Therefore in practice, much more glucose is required to provide enough electrons for all biological activities.

Current State and Future Trends of Sources Impacting Nitrate, Arsenic, and Selenium Releases in Mining

Mineral processing requires the processing of large quantities of ore. A major issue facing the mining industry is declining ore grades and the development of countries such as China and India and expected future demand for metals. Figure 5 depicts the rise in the flow of materials in the U.S. from 1900 to 2000. Resource depletion models can provide insight to

Table 7. The balanced oxidation half reactions for glucose, ethanol, and acetate based on hydrogen and carbon dioxide.

Glucose	$6H_2O + C_6H_{12}O_6 \rightarrow 6CO_2 + 24H^+ + 24e^-$
Ethanol	$3H_2O + CH_3CH_2OH \rightarrow 2CO_2 + 12H^+ + 12e^-$
Acetate	$C_2H_3O_2^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^-$

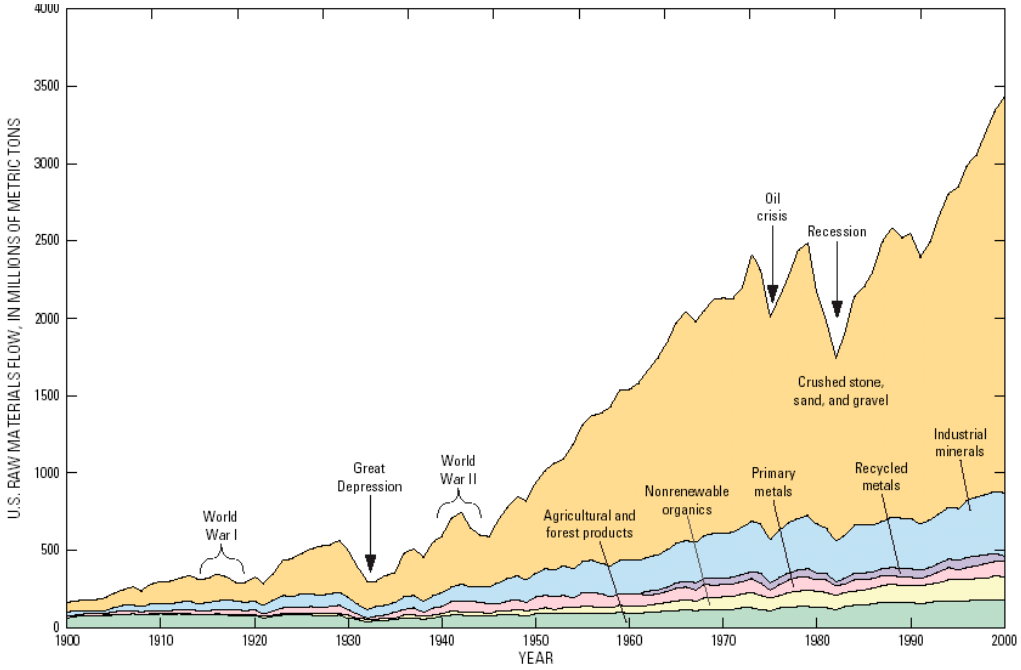

Figure 5. U.S. flow of raw materials by weight from 1900 to 2000 (72).

develop responses for the recovery of an increasingly difficult and important resource. Few researchers have explored the concept of resource depletion curves when applied to minerals; however, their work is compelling and “peak minerals” can serve as a metaphor in which to consider the future of the mining industry (73) (74). While most minerals are unlikely to be exhausted soon (75), they are becoming more difficult and costly to produce due in large part to diminished ore grades (76). The impact of declining ore grade will increase mine wastes and the corresponding environmental footprint (77).

The Electro-Biochemical Reactor

The fundamental EBR principle is the reverse of the microbial fuel cell (MFC) concept, where microbes transfer electrons to electrodes. The concept of MFCs have been around since 1911 when M.C. Potter first performed work on the subject (78) and later in the late 1970s when the concept of mediator-less MFCs was developed (79). An example of an MFC can be seen in Figure 6. An MFC is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganisms (80). Electrical energy is generated by the microbes from a carbon source such as glucose and in the absence of oxygen; CO_2, electrons, and protons are the products produced and shown in Table 5. A typical MFC consists of anodic and cathodic chambers separated by a cation specific membrane and is shown in Figure 6. In a mediator-less MFC, microbes will typically contain redox proteins such as cytochromes on the outer membrane that can directly transfer electrons directly to the anode (81) (82).

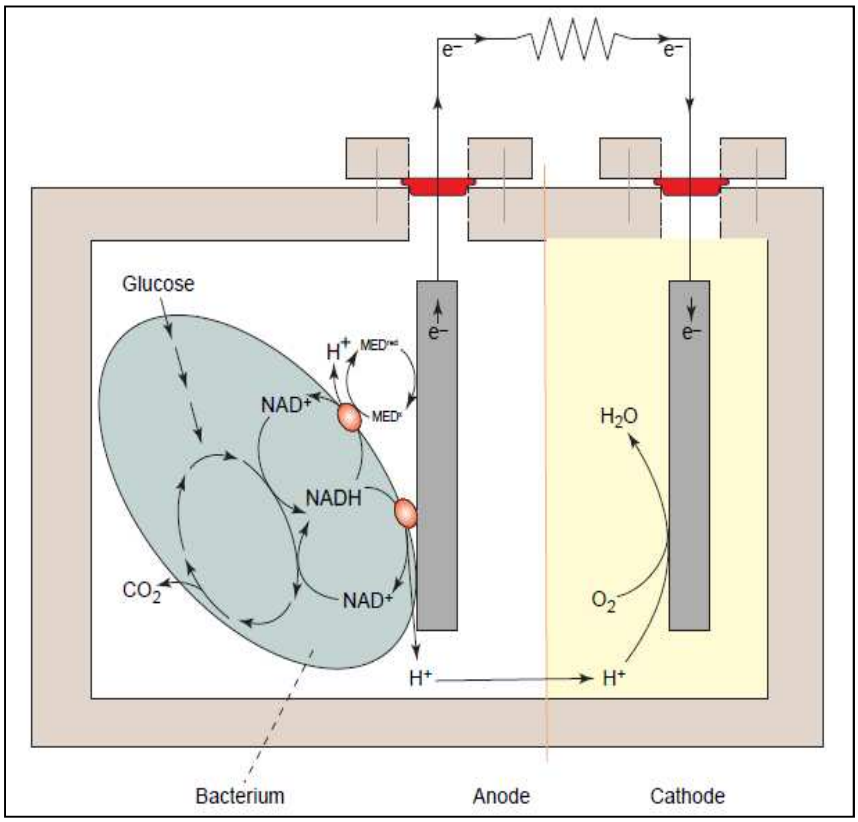

Figure 6. Simplified schematic of a microbial fuel cell. Substrate (glucose) is metabolized by bacteria and then gained electrons are transported to the anode (83).

The EBR technology improves upon conventional biological treatment methods, overcoming their shortcomings by directly supplying excess electrons to the reactor and microbes, using a low applied voltage across the reactor environment (6). In most cases, 1 to 3 volts DC and 1 to 5 μA is all that is required for the EBR, noting that 1 μA supplies $6.24x10^{15}$ electrons every second. These electrons replace the electrons normally supplied by excess nutrients and chemicals, resulting in considerable savings and additional benefits, remembering that one mole of glucose provides only 24 electrons. The understanding of how microbes interact with electrons allows for a unique control mechanism on activity and redox potential in the bacterial environment.

The EBR technology is especially well suited for the removal of nitrate, arsenic, and selenium which are difficult to remove to ultra-low levels using conventional biological, chemical, or physical treatment methods (84) (85) (86) (57). The EBR system overcomes these issues by providing readily available electrons for microbial growth and contaminant removal, resulting in better performance in less time and space and with greater efficiency.

The provided electrons result in a more controllable, economical, and robust system in comparison to past generations of conventional biological treatment systems. A small solar grid, batteries, or converters can easily supply the electrons needed for a full-scale facility.

The first successful EBR lab-scale test using a biocathode as the only electron donor for biological reactions was achieved in 2007 (87). In this particular investigation, the energy supplied to the cathode was obtained from the oxidation of acetate in the anodic chamber of the MFC separated by the cation exchange membrane. Although this particular study coupled organic removal, power generation, and denitrification into one system, the results clearly show that an applied cathodic voltage was able to sustain denitrification and increased denitrification kinetics by microbes on the electrode.

The fundamental research regarding the reduction of metals and inorganics, specifically arsenic, selenium, and nitrate using EBR principals that transfer electrons from an electrode to the bulk of the biofilm developed in bioreactors and other systems, has not been published as of the time of the writing of this thesis. This information is proprietary due to developed and

developing EBR patent applications involving both microbial-mediated reduction and oxidation reactions that are applicable to most wastewater treatment applications and many other applications including enzyme system reactions, but is presented in general EBR concepts.

Research for the removal of U (VI) using a graphite electrode inoculated with known metal-reducing microbes versus a standard graphite electrode suggests the reduction of U (VI) to U (IV) (88) (89). The application of a biocathode in this particular study was proven to be able to carefully maintain the conditions to promote the activity of the appropriate metal-reducing microorganisms near the cathode. It was shown that the same metal-reducing microorganisms grown in the absence of an applied voltage quickly declined and sulfate-reducing microbes became the primary organism.

While the understanding of the MFC has been studied for over 100 years and the body of research as to the mechanisms of electron transfer is well documented, the same cannot be said for the interactions between the cathode and the microbe. The important question for this thesis is whether microorganisms can uptake electrons from the cathode for use in the reduction of nitrate, arsenic, and selenium. The direct or indirect transfer of electrons to the microbe or to microbial biofilms for electron donor reactions is just now beginning to be considered and the fundamental research as to this phenomenon, although not well understood, suggests several mechanisms. By elucidating the mechanism through which electron transfer, from the cathode to the microbe, takes place, a strategy can be developed for optimization and application to bioreactors for reduction and oxidation processes.

The primary mechanisms suggested include direct cytochrome uptake of free electrons, intermediates such as humic acids, and pili type structures with enhanced electrical abilities. Each potential mechanism provides strategies under which individual microbes or a complete biofilm can efficiently and effectively provide energy for metabolic processes. Each mechanism is reviewed below.

Direct Electron Transfer to Microbes via *c*-Type Cytochromes

As discussed in the previous section, direct electron transfer from the microbe to the anode has been well documented in MFCs. Even now, recent research has documented and characterized a chain of *c*-type cytochromes capable of transferring electrons from the microbe across the cell wall to extracellular electron acceptors (i.e., anodes) (90) (91). However, research into the transfer of electrons from the cathode to the microbe is limited, but evidence exists everywhere in nature. One example is the oxidation of iron (II) and sulfur in acidic environments via microbial-mediated reactions (acid mine drainage). Several experiments have been carried out that identify specific cytochromes that are capable of accepting electrons directly from Fe (II) minerals (i.e., pyrite) and transferring this electron through the electron transport chain with oxygen reduction as the final reaction step (92) (93) (94). This research developed in support for direct cytochrome uptake of electrons focused only on oxygen as the terminal electron acceptor and is shown in Figure 7(A).

In the previous studies, the transfer of the electron was shown to be conserved through the production of ATP. This research suggests that for the cathode to act as an electron donor, the outer membrane cytochromes have to pass electrons to a more electro-positive electron acceptor within the periplasm and inner membrane of the microorganism. This interpretation of the results would require cytochromes to be able to take up a broad spectrum of electrons with different energies for the reduction of different inorganic compounds, as noted by the half reaction potentials in Table 6.

Mediated Electron Transfer

The ability of microorganisms to transfer electrons via an extracellular path, through insoluble electron acceptors such as iron-or manganese-oxides or inert electrodes, has long been exploited in MFCs. However, the study of mediated electron transfer from the cathode to the microbe has been largely unexplored. Recently pursued by several researchers (94, 95, 96), the use of redox mediators (humic acid, methyl viologen, etc.) and their effect on chemical end products provide initial evidence of possible electron acceptor pathways within microbes. Redox

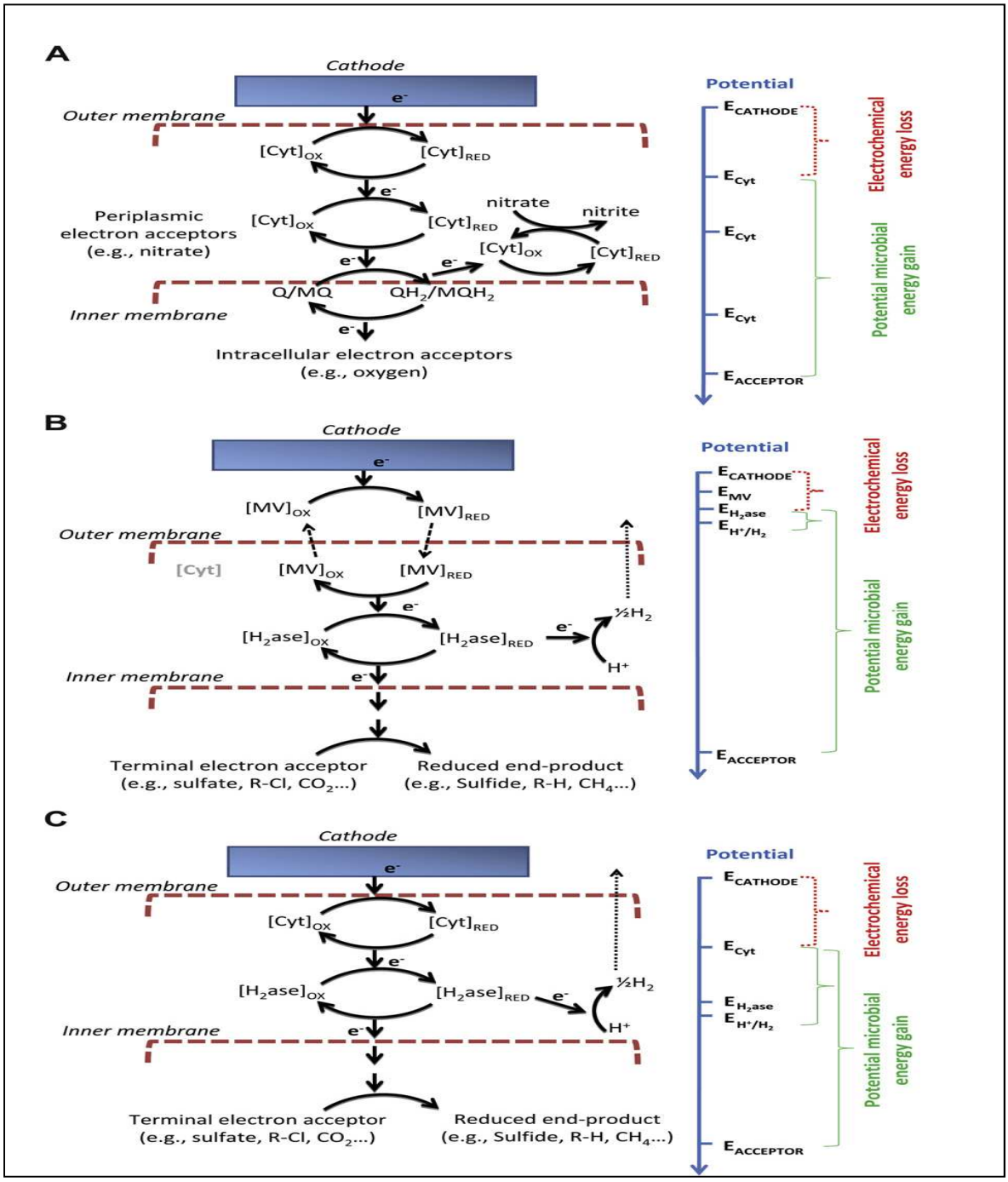

Figure 7. Proposed cathodic extracellular electron transfer mechanisms and associated energy gains for biocathodic microorganisms: (A) Direct electron transfer involving c-type cytochrome electron transfer chains. (B) Mediated electron transfer to a periplasmic hydrogenase. Examples are given for terminal electron acceptors; the reaction site for the terminal reduction depends on the type of electron acceptor. (C) Direct electron transfer involving cytochrome-hydrogenase partnerships (90).

mediators posses the advantage of known redox and chemical properties, thereby narrowing down the electron/microbe interaction pathway. To explore the role of redox shuttles, the previous studies mentioned used methyl viologen (MV) as the artificial electron shuttle, shown in Figure 7(b). MV has a standard redox potential (-446 mV) closely associated with H_2 (-414 mV) and can be employed as a redox partner for hydrogenasis. Hydrogenase is an enzyme produced within the microbes cytoplasm and electrons entering the metabolism directly at the hydrogenase level, shown in Figure 7(c), are expected to provide no energy to the microorganism (95) (96). Furthermore the properties of MV have been well documented with respect to microorganisms and their permeability across different membranes, i.e., outer and periplasmic membranes. It has been shown that MV cannot penetrate the cytoplasmic membrane (97) (98). Coupling those results, researchers have drawn the conclusion that direct electron transfer from MV to hydrogen across the cytoplasmic membrane is the most likely pathway.

Electron Transfer through Immobilized Structures

Thus far, the discussion of cathode/microbial electron transport has focused on intrinsic (cytochrome) and natural (MV) mediators. However, a third method of electron transport has been observed in MFCs and has gained the attention of several studies in cathode/microbial electron transport. In the case of MFCs, several species of bacteria (*Geobacter* and *Shewanella*) exhibited conductive structures, termed nanowires, that were necessary for the reduction of Fe(III) oxides (99) (100). Direct electron transport is possibly bidirectional, as *Geobacter* has shown to accept electrons from a cathode (101). The importance of bacterial nanowires may be that they facilitate electron transfer from bacteria to metal oxides and from electron donors (cathodes) without the need for direct cell/mineral or direct cathode/cell contact or the use of dissolved electron shuttles (102). Furthermore, nanowires can facilitate electron transfer and energy distribution within the biofilm, providing a more efficient method for electron donor/acceptor reactions (103).

MATERIALS AND METHODS

EBR Bench-scale Testing

Bench-scale testing is necessary to establish design and operational boundaries for removal of contaminants from wastewaters; this is especially true for diverse mining waters containing various levels of nitrate, arsenic, and selenium and other site-specific contaminants. Bench-scale EBR testing provides evaluation and optimization of the EBR system processes for a specific water to be treated. This includes the microbes involved, system design, electron voltage potential, testing procedures, nutrient requirements, sampling and analysis, and evaluation for pilot-scale testing.

Water Sample Collection

The first steps in bench-scale treatability testing is evaluation of water chemistry, to determine if pre- and posttreatment steps will be involved, and obtaining a representative sample of water to be treated. Co-contaminants affect treatment system design and operation. Water sampling points are selected such that water samples collected are representative of nitrate, arsenic, and/or selenium concentrations and other water chemistry components normally found in the system.

Testing conducted for the Gold mine water included the receipt of two 55-gallon drums of mine water collected by mine site personnel from a 400 gallon galvanized steel reservoir that semicontinuously overflows into the mine water impoundment basin. The steel reservoir is filled via a lift pump from a mine water pump-back collection point that receives water from the mine's east heap. Testing conducted for the Base Metal mine water included the receipt of four 55-gallon

drums of mine water collected by mine site personnel from the Mill pump-back tank located next to the mine water impoundment basin.

Both sets of samples included initial water analysis completed at the mine site as collected and both were reanalyzed upon receipt and at several times during the bench-scale test. All samples were shipped to the University of Utah, College of Mines and Earth Sciences, Department of Metallurgical Engineering, Ivor Thomas Lab and stored at room temperature. Once at the lab, the drums were stirred to produce a homogeneous solution, and two 1-gallon samples were withdrawn as needed for lab testing and analysis. Both mine water samples were used without pH adjustment. Analysis of nitrate, arsenic, and selenium were completed by an independent state certified water lab. Arsenic was also analyzed at the University of Utah, College of Mines and Earth Sciences, Department of Geology.

Water Contaminant Analysis

Analysis parameters required are dependent on the specific sites' water chemistry, system processes needed for contaminant removal, including the EBR process, and site contaminant removal goals or contaminant treatment targets. Water chemistry parameters and contaminant treatment targets for the Gold mine and Base Metals mine are given in Tables 8 and 9, respectively.

Gold Mine Water Target Contaminants, Influent Concentrations, and Discharge Targets

Table 8 provides the influent contaminate concentrations and the discharge targets for the specific contaminants for the Gold mine waters.

Table 8. Nitrate and arsenic influent concentrations and discharge targets; Gold mine.

Contaminant	Gold Mine Average Influent	Gold Mine Discharge Target
Nitrate- N (ppm)	24.3	<10
Arsenic, Total (ppm)	0.66	<0.05

Table 9. List of water parameters, average influent concentrations, and discharge targets; Base Metal mine.

Contaminant	Base Metal Mine Average Influent	Base Metal Mine Discharge Target
Physical Parameters		
pH (su)	8.15	6.5-9
TSS	9.23 ppm	15 ppm
Anions and Nutrients		
Ammonia-N	2.12 ppm	5 ppm
BOD	<10 ppm	25 ppm
Nitrate-N	2.16 ppm	10 ppm
Nitrite-N	0.26 ppm	0.6 ppm
Sulfate	530 ppm	1800 ppm
Cyanides		
Total Cyanide	0.18 ppm	0.05 ppm
WAD Cyanide	0.05 ppm	0.02 ppm
Total Metals		
Aluminum (Al)	0.16 ppm	0.8 ppm
Antimony (Sb)	0.08 ppm	0.08 ppm
Arsenic (As)	0.007 ppm	0.05 ppm
Cadmium (Cd)	0.003 ppm	0.002 ppm
Copper (Cu)	0.25 ppm	0.015 ppm
Iron (Fe)	0.42 ppm	0.5 ppm
Lead (Pb)	0.13 ppm	0.02 ppm
Mercury (Hg)	0.00004 ppm	0.001 ppm
Molybdenum (Mo)	0.047 ppm	0.73 ppm
Nickel (Ni)	0.008 ppm	0.5 ppm
Selenium (Se)	1.34 ppm	0.02 ppm
Silver (Ag)	0.003 ppm	0.001 ppm
Zinc (Zn)	0.18 ppm	0.5 ppm

Base Metals Mine Water Target Contaminants, Influent Concentrations, and Discharge Targets

Table 9 provides average influent contaminant concentrations and discharge targets for the Base Metal mine.

Analysis Techniques

Analysis of water chemistry parameters are detailed in many standard texts, e.g., “Standard Methods for the Examination of Water and Wastewater.” The following tables list the parameters tested, analysis technique, and equipment and EPA standard method used to collect water quality parameter data. Table 10 lists certified lab methods and Table 11 lists parameters and equipment used to collect in-house and field data measurements.

Quality Control Techniques

During bench- and pilot-scale testing, quality control samples included samples collected and sent as duplicates and samples collected and diluted with de-ionized water to ensure accurate nitrate, arsenic, and selenium results. Quality control samples were taken at least once a month unless major discrepancies in results were observed.

Table 10. Certified lab parameters tested, and equipment employed and EPA method.

Parameter	Equipment	EPA method
Ammonia -N	Hach Corp TNT reagent vials and DR 800 Colorimeter	Method 10205
Biological Oxygen Demand (BOD)	Please refer to appendix	Method 5210 B
Cyanide	Colorimeter	Method 9012A
Cyanide WAD	Colorimeter	Method 9012A
Nitrate-N, Nitrite-N	Ion Chromatography (IC)	Method 300.0
Metals (total and dissolved)	ICP-MS	Method 200.8
Sulfate	Colorimeter	Method 375.2
Arsenic	ICP-MS	Method 200.8
Selenium	ICP-MS	Method 200.8

Table 11. Field parameters tested, equipment employed, and standard method.

Parameter	Equipment	Method
Flow rate	3 liter graduated container, stop watch	SESDPROC-109-R3
ORP	Thermo Scientific brand meter, platinum redox electrode, and silver/silver chloride reference electrode	SESDPROC-113-R1
pH	Thermo Scientific Accumet AB15 pH meter, Accumet pH probe 13-620-111 SN 1350027P7	SESDPROC-100-R3
Temperature	Thermo Scientific brand meter, stainless steel probe	SESDPROC-102-R3
Dissolved Oxygen (DO)	Orion 3-star DO portable meter, Orion 083010MD DO probe	EPA method 360.1
Chemical Oxygen Demand (COD)	Hach DR/890, Hach Digester DRB200, Hach TNT COD vials.	EPA method 410.4

To minimize discrepancy between duplicate samples, samples were collected in a single container, which was then used to fill duplicate sample bottles. For diluted samples, collected water samples were measured and/or filtered into an appropriate graduated glass cylinder and de-ionized water was added to achieve the desired dilution. The sample was then poured into a prewashed lab supplied sample bottle, appropriate preservative added, and sent for analysis. During bench-scale operation, all samples tested for arsenic and selenium were sent to an independent laboratory and/or the Department of Geology at the University of Utah for ICP-MS analysis. Dilution series consisted of samples diluted 1:2, 1:4, and 1:10.

Analysis of results to establish statistical significance was employed where discrete values were obtained using the analytical methods described in Tables 3 and 4. Where results were compared, a standard z-value was calculated and the probability of rejecting the null hypothesis was set at 5%.

Microbial Evaluation

The fundamental mechanism of nitrate, arsenic, and selenium removal in the EBR system is mediated through microbial electron donor/acceptor reactions, and redox reactions. Microbial evaluation was based on two criteria; 1) microbial growth rate and 2) microbial contaminant removal. Different microbes chosen for a specific mine water were grown in tubes containing a standard 30 g/L concentration of Trypticase™ Soy Broth (TSB). TSB supports growth of a wide variety of aerobic, anaerobic, and facultative microorganisms commonly used in bioreactors. Once the microbes reached a high population density, based on turbidity, they were screened using a 1:2 dilution in the specific mine water under three different conditions: 1) mine site water spiked with 25 mg/L of the target metal and 30 g/L TSB; 2) mine site water spiked with 5 mg/L of the target metal and 30 g/L TSB; and 3) mine site water with 30 g/L TSB.

Microbial samples chosen for evaluation for the Gold mine were As^{-1}A, As^{-1}B, 1B, 2B, Pseudomonas Denitrificans, and Bacillus Subtilis.

Microbial samples selected for evaluation for the Base Metal mine water were As^{-1} B, SeO4 Br, LORAX, ZO, SF33E, SF33F, SF33F ISO, SF33E, SF33A, SF33A, SF33B, SF33G ISO, Sf33E ISO, SF33D, $SeMix^{1D}$- ISO, SF005a, Se 24^{-1}-A, Se 24^{-1}-B, Se 24^{-1}-C, Se 24^{-1}-D, Se 24^{-1}-E, Se 2uF, Se 2D, Se 2uA, Se 2uB, Se2uC, Se 2uD, Se 2uD-ISO, Se MixA, Se MixB, and Se MixB-ISO.

Microbial Growth Rate

Microbial growth rate was assessed based on light absorbance. Sample light absorbance at 600 nanometers (OD_{600}) was related to the number of bacteria on a sample plate using standard sample dilution and microbial plating techniques. Growth was determined using the different microbial cells diluted to an initial OD_{600} value of between 0.2 and 0.4 in a 1 liter flask mixed on a magnetic stir plate at room temperature. Samples were taken from the flask every 2 hours for 24 hours and the absorbance reading recorded. An aliquot of each sample, measured on the spectrophotometer, was also diluted using 10-fold dilutions to concentrations of 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} and placed on TSA plates to determine the number of microbial colonies at

each dilution. Graphs were constructed with the abscissa (x-axis) as time and the ordinate (y-axis) as OD_{600} absorbance. Population density under different growth conditions, based on absorbance values, were compared and evaluated with microbial plate count data.

Contaminant Removal Determinations

The ability of a particular microbe or microbial population to remove or reduce target contaminants from water samples was determined based on two methods. The first method tested a sample of water from each tube for the target contaminants concentration. Water samples were tested initially and then at 2-hour intervals for the first 10 hours; Samples were subsequently examined at 24 and 48 hours. Water samples were filtered through a PES (Polyethersulfone) membrane filter with 0.45 µm pore size, preserved using nitric acid for metals, or other appropriate preservative for other analytes, and sent to lab for analysis.

The second method subjectively ranked sample tubes containing microbes and selenium as in Figure 8. Three sample tubes were prepared for each microbe to be screened. Sample tubes were filled with mine water with two of the sample tubes spiked with 2 mg/L and 25 mg/L of selenium-selenate. The ability of the microbes or microbial population to grow and reduce the target contaminant in mine water, at the native pH, was visually quantified and evaluated based on color, amount of precipitate formed, and turbidity over 10 days. All screening tubes were ranked visually at 2, 5, and 10-day time intervals and a graph was developed.

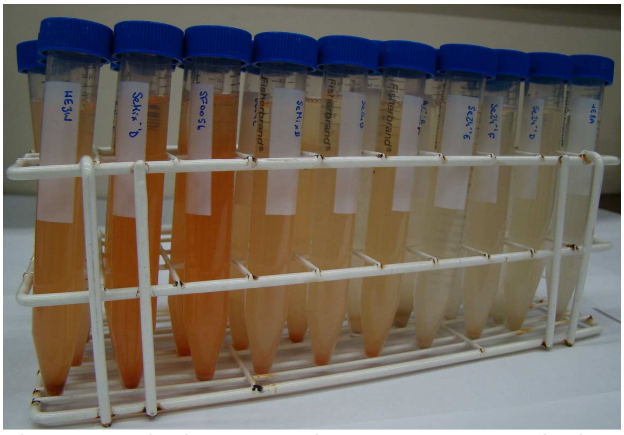

Figure 8. Selenium screening test, Base Metal mine.

MSM

Bench Testing

MSM is used to provide a microbial attachment surface and high surface area able to support high population densities. Support materials tested included granulated activated carbon (GAC), pumice rock, and scoria rock. GAC included both 4 mm pelletized and 8 x 40 sieve size. GAC is typically made from coconut shells and/or bituminous coal. Pumice rock and scoria rock sizes were 0.5” to 0.25" in diameter. GAC and pumice rock are porous and scoria rock is a solid. MSM material was washed prior to use in the bioreactors to remove dirt and debris. Washing consisted of rinsing with a 1 N HCL solution followed by rinsing with de-ionized water until rinse water pH indicates no further influence by the HCL solution, or reaches a pH >6. The MSM is then oven dried.

Pilot Testing

The pumice rock for the pilot-scale test Gold mine was 1.5" to 0.5" in diameter. Pumice rock was delivered to the mine site and rinsed with water from the 400 gallon galvanized steel reservoir used for EBR system influent and allowed to dry in the sun on a plastic tarp before being loaded into the EBR tanks. GAC for both the Gold mine and Base Metal mine used 8 x 40 sieve size. GAC was rinsed in 55 gallon drums with bottom drain valves. Each drum was filled with a 1 N HCL solution and allowed to soak for 24 hours. The drums were then drained and rinsed using drinking water until a pH >6. The GAC was then dried on a plastic tarp in the sun and loaded back into the 55 gallon drums and shipped to the job site in the system container.

Nutrients

Bench Testing

Each EBR bioreactor was fed once every 24 hours in a 15-minute pulse. 100 ml of effluent water from each EBR was used as make-up water for that EBR. Trypticase soy broth (TSB) at a standard concentration of 30 g/L or molasses at a concentration of 4 g/L was used for the first week of operation. Molasses concentrations ranging between 0.5 to 4 g/L were evaluated

during EBR operation and the concentration used was dependent on the testing phase and the wastewater chemistry being examined. Phase 3 of EBR operation was the nutrient stressing phase where molasses concentration was reduced in a step wise fashion until nitrate, arsenic, and selenium removal targets were no longer removed to acceptable levels. Effluent water from each EBR was used to dilute the nutrient to the desired concentration. EBR effluent pH ranged from 5.5 to 7 and no pH adjustment was used for nutrient make-up water.

Pilot Testing

Nutrient for pilot-scale testing was animal grade molasses. Gold mine nutrient was made up in a 55 gallon drum using influent site waters. Nutrient was added once every 24 hours for the first 30 days and twice every 12 hours for the remainder of the test. 50 gallons of diluted nutrient solution was pumped to each reactor. Molasses concentrations ranged between 1 and 5 g/L. Nutrient addition for the Base Metal mine was made in 5 gallon buckets using effluent water from each EBR. Nutrient was added once every 24 hours. Molasses concentrations ranged from 0.75 to 4 g/L.

EBR Bench-scale System Reactor Design

Standard wastewater treatment systems incorporate several process steps to achieve final effluent discharge targets. Process steps include equalization, primary treatment, secondary treatment, and tertiary treatment. Waste by-products such as solids or contaminated concentrated wastewater are also addressed in overall wastewater system design. The EBR process provides treatment options for both influent wastewater and/or residual wastewater treatment by-products. Figure 9 provides an overall wastewater system design process schematic and where the EBR system components reside relative to the potential components used in a possible configuration with all treatment components considered.

Design of the EBR system in this thesis was for site-specific water treatment and the systems were tailored to remove nitrate, arsenic, and/or selenium. The ability of the EBR system to achieve target contaminate removal at pilot- or full-scale may require all or part of the process

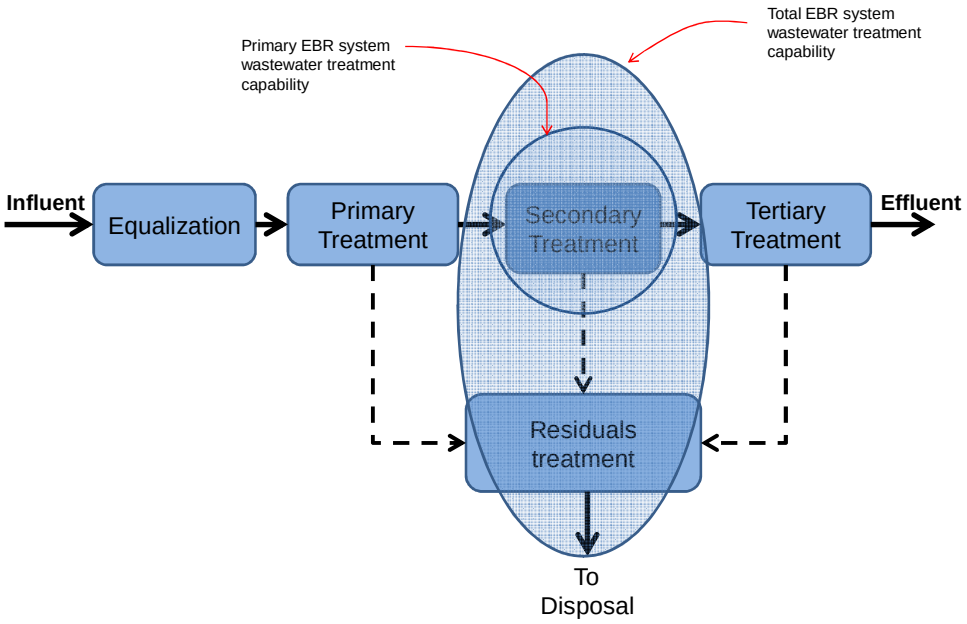

Figure 9. Overall wastewater treatment process schematic.

steps described in Figure 9. Water chemistry and nitrate, arsenic, and selenium concentrations are different at each mine site. Therefore, EBR and system requirements such as EBR size, electrode placement, number of pre- and post-EBR process steps, HRT, electron input, and nutrient requirements will vary from bench- to pilot-scale. Bench-scale testing was used to determine HRT, electron input, and nutrient concentration influence on EBR performance.

EBR Bench-scale Construction and Materials

The bench-scale EBR reactor was constructed using standard plastic plumbing parts and fittings purchased from commercial plumbing stores, as shown in Figure 10. The main body of the EBR reactor was a 3” diameter clear PVC (Polyvinyl chloride) plastic pipe 16” long. Medical grade plastic tubing from the peristaltic pump was used to introduce water and nutrients to the reactor bottom. At the reactor top, tubing was used for effluent gravity-flow discharge.

Figure 10. Example of EBR bench-scale reactor test systems. The EBR on the left allows electron input at different points. The EBR right is an example of a single electron input point.

Sample ports were included in the bench-scale EBR reactor design at different points, corresponding to different HRTs, for evaluation of contaminant removal effectiveness and electrode influence. As an example, to examine the effect from electron additions, a pipette was inserted to a depth just above the cathode and a second pipette was inserted to a depth just below the anode. Placement of the cathode and anode is somewhat dictated by the length of the reactor, but is designed to provide additional electrons as the nutrient supplement provided electrons, based on metabolism, are decreasing. Electrode placement is designed to maximize the time the influent water is influenced by the electron field.

Electrode material for the cathode was ¼" diameter stainless steel rod 6" long. Electrode material for the anode was ¼" diameter titanium rod 6" long. Electrodes were attached to the reactor using plastic threaded compression fittings. Electrodes were positioned inside the reactor perpendicular to the water flow path. A Mastech brand direct current (DC) power supply with 0-30V and 0-3A continuous adjustable output was attached to the electrodes and used to regulate voltage - amperage input using standard crimp and screw type electrode connectors. Reactor voltage was evaluated at bench-scale and used appropriately at pilot-scale.

EBR Bench-scale Operation

EBR system bench-scale operation was divided into five periods or phases. Each phase was designed to establish operational boundaries for pilot-scale design and testing. The five phases are as follows:

- Phase 1 – Inoculation. Establishment of the selected microbial consortium within the reactor was completed by filling the bioreactor with inoculum and allowing it to stand undisturbed for 72 hours; this provided time to establish initial microbial biofilm development on the MSM surfaces. Mine water to be treated was then pumped into the reactor at a flow rate equivalent to a 24 hour HRT
- Phase 2 – Contaminant Removal Validation. Pre- and postprocess steps are added, removed, and changed as data become available on performance.
- Phase 3 - Nutrient Stressing. Examination of variations in the C:N:P balance and concentration.
- Phase 4 – Process Stressing. Examination of each process step to its operational limit – beyond its ability to meet discharge targets with a minimum HRT.
- Phase 5 – Process Recovery/Optimization. Stressing the EBR system helps define system operation optimization parameters examined during this phase.

Influent Feed

A 1-gallon plastic bottle was used for the EQ or influent feed tank; this bottle was washed weekly. Bench-scale influent water samples are acquired from stirred 55-gallon drums. Addition of water, nutrients, and chemicals to different EBR stages or process components was accomplished using a variable speed peristaltic pump. EBR bench-scale systems all had an influent flow rate of 1 liter per day. Pump speeds were physically measured during testing using a graduated cylinder and stop watch for verification and flow rate adjustments. Samples for analysis were collected before any adjustments were made to the peristaltic pump. Nutrient and/or chemical additions were typically introduced into their respective addition points using the same peristaltic pump and opening or closing appropriate ball valves.

EBR Bench-scale Design and Operation - Gold Mine

EBR bench-scale system design and operation for removal of nitrate and arsenic from the Gold mine waters used a single EBR reactor with no pre- or postprocess steps. Additionally, a CBR (no voltage) reactor was constructed using the exact same design as the EBR. MSM materials were a combination of pumice rock and GAC. EBR electrodes were sandwiched in a layer of GAC. Voltage to only the EBR was increased from zero volts, to 1 volt, to 3 volts in step-wise manner. Both the EBR and CBR nutrient was a proprietary molasses blend; a 24-hour HRT was used during this evaluation. Figure 11 is a schematic of the EBR bench-scale system for the Gold mine.

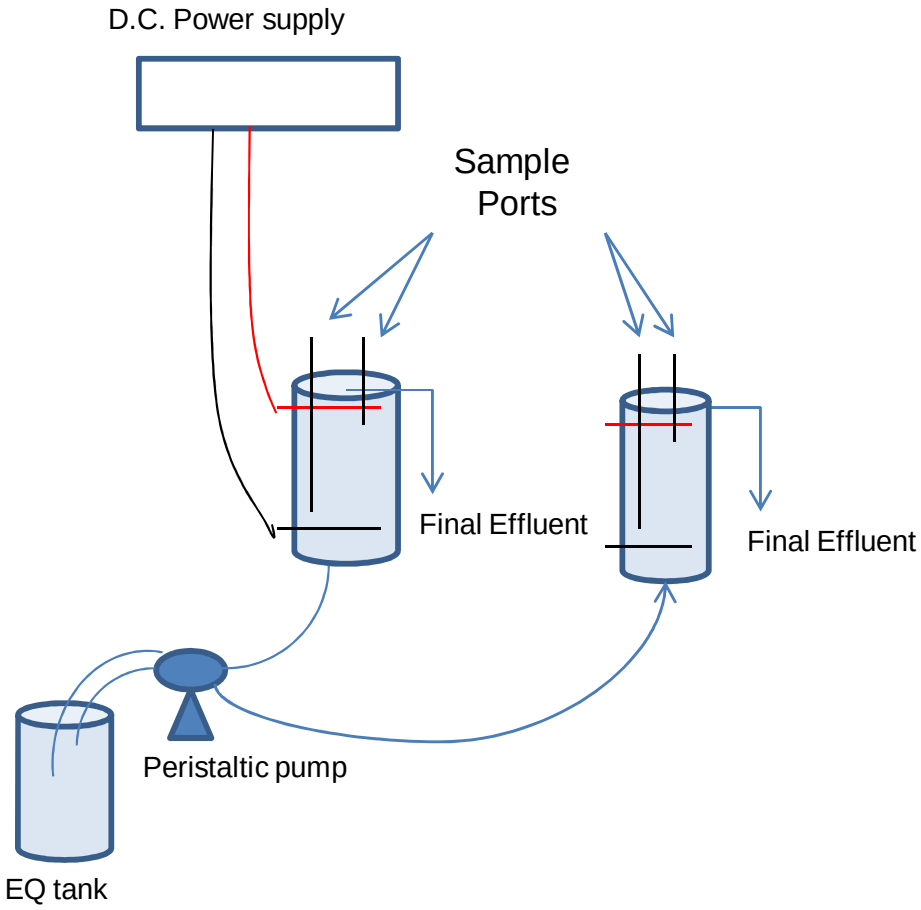

Figure 11. EBR bench-scale flow schematic, Gold mine.

EBR Bench-scale Design and Operation - Base Metal Mine

The Base Metal mine EBR bench-scale system was designed and operated for the removal of selenium. Two pretreatment processes were examined for the removal of residual flotation reagents used in the ore milling process. Posttreatment was required for removal of residual BOD. Process steps examined included:

- Pretreatment using activated carbon (AC) - A carbon absorption step. COD was measured before and after the AC column to identify breakthrough of residual flotation reagents. The EBR reactor followed the pretreatment column.
- Pretreatment using anaerobic bioreactor- Microbial COD degradation. COD was measured before and after the anaerobic pretreatment step to examine residual flotation reagent degradation. The EBR reactor followed the pretreatment reactor.
- EBR Reactor. The EBR was operated as a dual stage with a 24-hour total retention time. The MSM used for the EBR column was GAC.
- Posttreatment. Posttreatment consisted of two steps for removal of any excess BOD and aeration of effluent water. The first posttreatment step was an anaerobic biological step; GAC was used as the MSM. Following the anaerobic step was an aerobic bioreactor. The aerobic step acted as a polishing step for the anaerobic process and reintroduced oxygen into the discharge waters to the required level, 6 to 7 ppm. Pumice rock was used as the MSM and the HRT was 8 hours. Figure 12 is a schematic of the Base Metal mine EBR bench-scale system.

EBR Pilot-scale System Reactor Design

For each mine water tested, a site-specific EBR system component size and configuration was used. However, several overriding design issues were taken into account as treatment criteria were addressed. Pilot-scale design considerations included flexibility to add or remove process steps as the pilot-scale tests progressed and as data became available. The overall concept for the EBR pilot-scale system was to require only power and water from a specific mine.

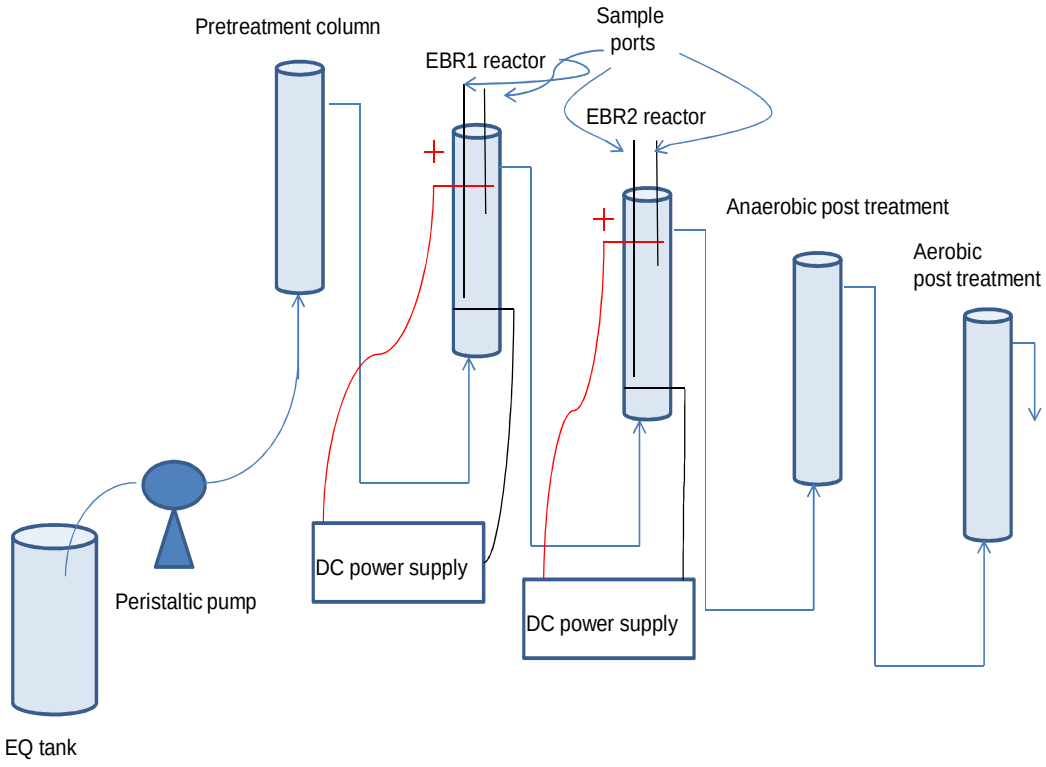

Figure 12. EBR bench-scale flow schematic; Base Metal mine. Example sample ports that were used in each reactor component are shown in the EBR system in this schematic.

The following issues were used as overall EBR pilot-scale system design criteria:

- System transport to and from a specific site
- Power requirements and sources
- System operation flexibility
- EBR support materials and equipment

The EBR pilot-scale system must be easily and safely transportable anywhere in the world to remote sites using various truck, rail, and/or ship modes of transport. Ease of loading and unloading the system took high consideration. As mine site waters vary greatly from site to site and between bench- and pilot-testing, process steps flexibility is an important design criteria. Based on the above, a 20’ L x 8’ W x 8’H shipping container was chosen, as shown in Figure 13, and equipped with a 480 VAC, 50 A, 3Ø transformer as this was the most common accessible power among the remote mine site locations reviewed. A 20’ shipping container also provided

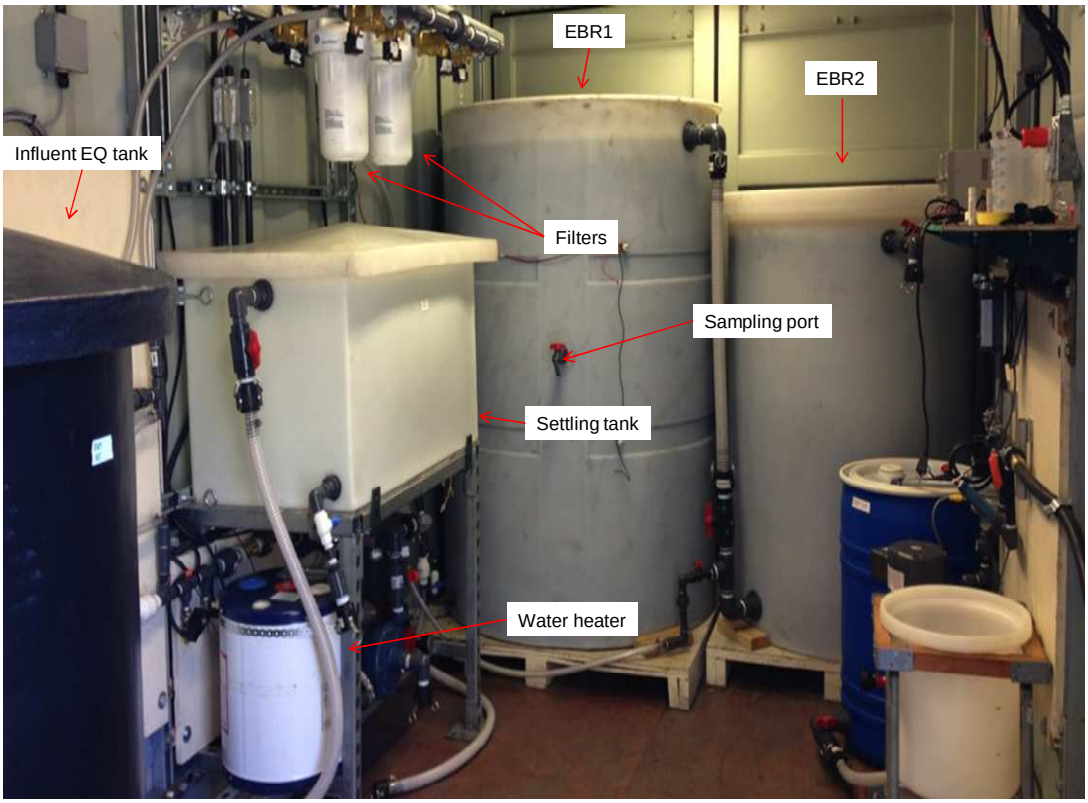

Figure 13. EBR pilot system.

space needed for all components as well as tie down points to secure all equipment so shifting and breakage would not occur during transport. Successful start-up and testing of the EBR pilot-scale system requires support materials and equipment, including microbial inoculum, pumps and plumbing, nutrient and chemical amendments, process tankage, MSM, and analysis equipment. A shipping container provides the space required for all equipment as well as tie down points.

EBR Pilot-scale Design and Operation - Gold Mine

Testing of the EBR pilot-scale system at the Gold mine represents the first EBR technology scale-up from bench (1 LPM) to pilot (4 LPM). MSM chosen was pumice rock with electrodes sandwiched in between a GAC layer. Based on bench-scale testing, a two-stage EBR in series; single pass; up-flow design was implemented and is shown in Figure 14.

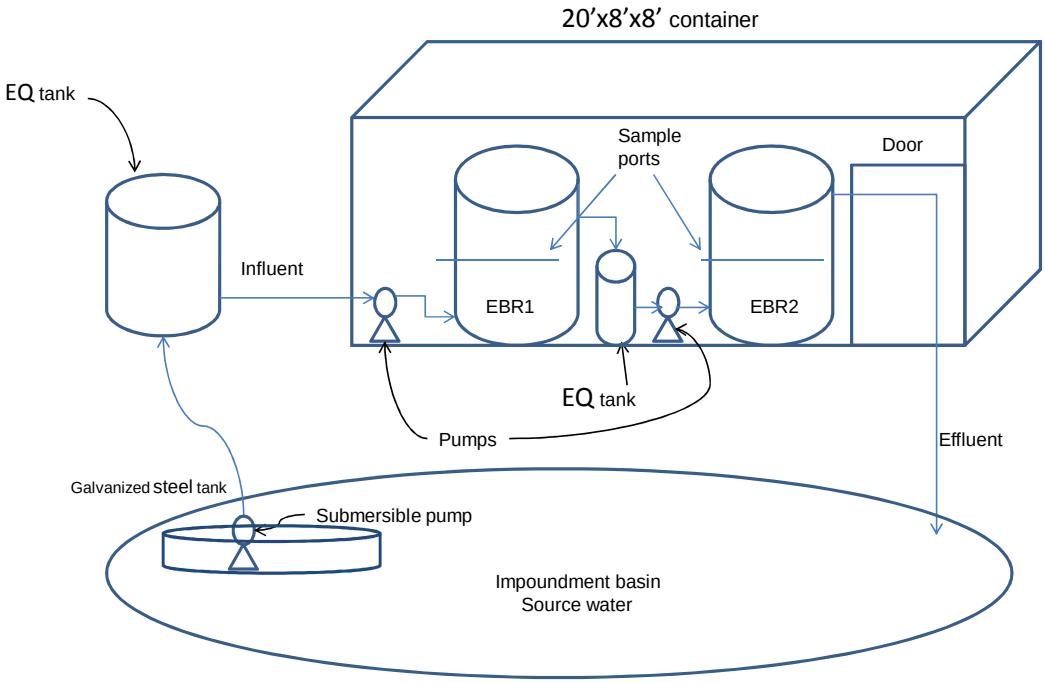

Figure 14. EBR pilot-scale system flow schematic; Gold mine.

EBR pilot influent water was pumped to a clean 1,135 liter plastic EQ chemical tote via a submersible pump. Water was pumped from the EQ tank to the first EBR reactor via a centrifugal pump. A flow meter directly followed the centrifugal pump and flow rate was adjusted manually using a plastic ball valve. The bottom of both EBR reactors contained a distribution system to allow for even distribution of water through the reactor to produce plug flow conditions. EBR electrodes for tank one and two were configured the same. Three stainless steel cathodes and anodes were used per system to deliver the electrons to EBR reactors. A Mastech brand direct current (DC) power supply with 0-30V and 0-3A continuous adjustable output was attached to the electrodes and used to regulate voltage - amperage input using standard crimp and screw type electrode connectors. Voltage input was set at 3 volts for the entire test. The cathodes were placed one foot up from the bottom of the tank and separated by 120^0 degrees. The anodes were placed 6" DOWN from the discharge point of the tank in the same configuration as the cathodes.

EBR operation was continuous following inoculum development. Influent water used for the Gold mine EBR pilot was taken from the exact same point as the water collected for the Gold

mine bench-scale test. Pilot-scale operation required manual operation of flow balance, nutrient addition, and voltage adjustment. Flow balance and adjustment was controlled via a ball valve and rotometer; system performance was evaluated from lab certified data. Nutrient addition was performed once a day for the first 30 days at a concentration of 5 g/L. From day 31 to the conclusion of the test, nutrient was added twice a day at 12-hour intervals over a range of 1 to 4 g/L. Voltage was set at 3 volts for the entire test. System samples were collected for independent lab analysis three times a week and on-site field measurements were collected from the following points everyday:

EQ tank discharge

EBR 1 effluent

EBR 2 effluent

Data collected included:

Alkalinity

Ammonia

Arsenic(Total and Dissolved)

Bicarbonate

BOD

Chloride

CO_2

COD

Flow rate

Iron

Manganese

Nitrate

Nutrient addition

ORP

pH

Phosphate-ortho

Sulfate

Temperature

Total Dissolved Solids

Total Organic Compounds

Total Suspended Solids

EBR Pilot-scale Design and Operation - Base Metal Mine

The EBR pilot-scale design for the Base Metal mine waters was developed for the removal of selenium; the design flow rate was one Lpm with a 24-hour EBR process HRT. Pilot-scale testing was completed over two summers and adjusted between the first and second year

to meet changing water chemistry treatment needs. High concentrations of residual mill flotation reagents present in the influent required the addition of an AC pretreatment step the first year and an anaerobic pretreatment step in the second year, as shown in Figure 15.

A water heater was used to heat the ~4^0 C influent water to ~20^0 C; it was not operated during the EBR temperature stress testing phase. The EBRs used GAC as the MSM. A separate Mastech brand DC power supply was used to apply 3 volts potential to each EBR reactor. EBR electrodes for tank one and two were configured the same. Three stainless steel rods were used as cathodes and three titanium rods were used as the anodes in each EBR. The cathodes and anodes were placed in the same plane. The cathodes were one foot up from the bottom of the tank and separated by 120^0 degrees. The anodes were placed 6" DOWN from the discharge point of the tank. Standard crimp-on and screw type connectors were used to connect the electrodes to the power supply.

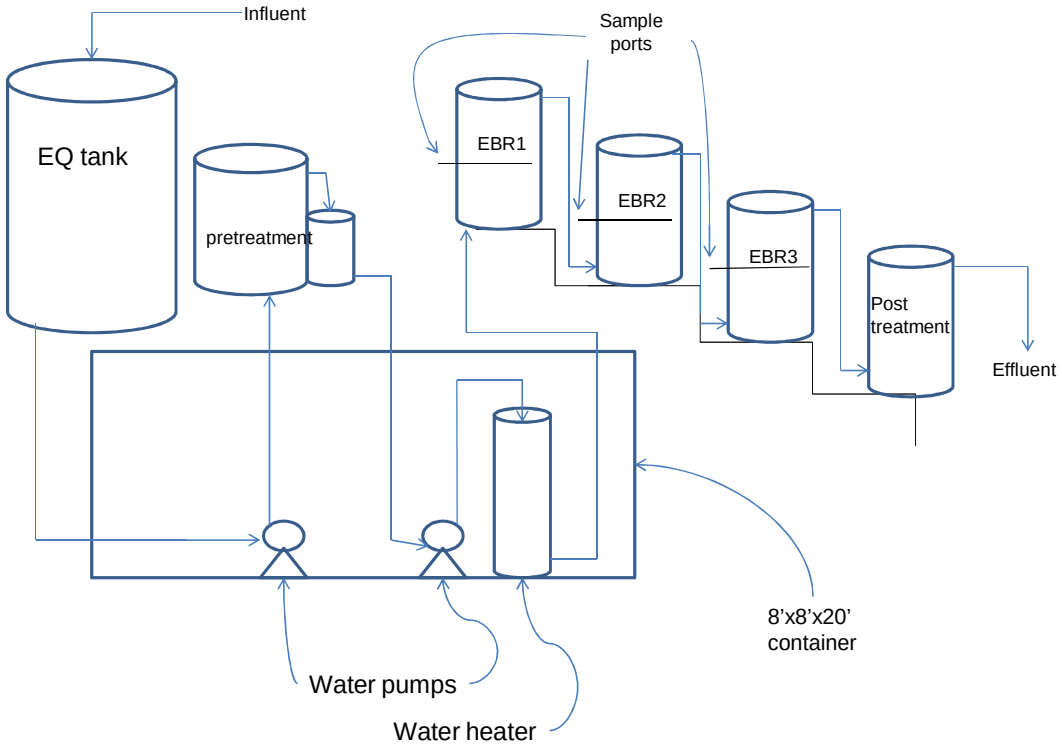

Figure 15. EBR pilot-scale system flow schematic, Base Metal mine 2011.

Year One

During year one of pilot testing, water was collected in an 18,925 liter tanker truck. Water was collected from two points: the flotation circuit water make-up tank located in the mill and the mill pump-back tank located near the water impoundment basin.

Molasses nutrient was added once a day at 0.5 to 4 g/L depending on the testing phase being examined. The EBR process was carried out in three tanks, each with an 8-hour HRT. MSM for the EBR used 8 x 40 sieve size GAC. The EBR tanks were connected in series and EBR 2 and EBR 3 were gravity feed.

Year Two

Changes made to the EBR system and system operation included the system EQ tank was continuously filled from the mill pump-back tank. An 8-hour HRT anaerobic biological pretreatment step with 8 x 40 sieve size GAC as the MSM was added to degrade residual flotation regents. Two EBR process tanks were used; EBR 1 had a 16-hour HRT and EBR 2 an 8-hour HRT.

Residual BOD from the EBR process was addressed in two stages. The first stage was a fixed bed single pass up-flow gravity feed reactor with an 8-hour HRT. The MSM material was GAC and inoculated with EBR 2 effluent. The second stage was a completely stirred aerobic reactor gravity feed from the anaerobic step. The aerobic reactor used pumice as the MSM and the HRT was 8 hours. Effluent was gravity feed to the mines water impoundment basin, as shown in Figure 16.

The Base Metal mine EBR system was operated continuously with manual adjustments of flow, nutrient addition, and voltage. Nutrients were added once a day. Nutrient concentrations ranged from 0.75 to 4 g/L. EBR system adjustments such as nutrient concentration and temperature were adjusted as parameter data became available from the water analysis laboratory. Nutrient addition consisted of weighing the molasses nutrient to achieve the desired concentration. Nutrient was added to each of the EBR reactors individually at 3 L/minute followed by increased flow at 3 L/minute for 3 minutes to push nutrient into the EBRs.

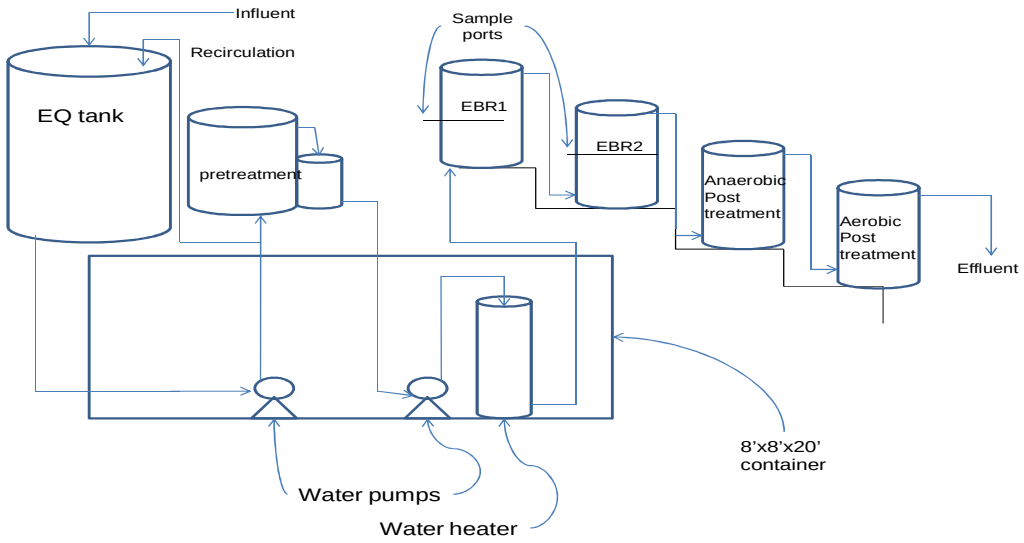

Figure 16. EBR pilot-scale system flow schematic, Base Metal mine 2012.

Sample points for the EBR system consisted of the following points:

Year one:	Year two:
Influent	Influent
Effluent EQ	Effluent precipitation tank
Effluent EBR-1	Effluent pretreatment tank
Effluent EBR-2	Effluent EBR-1
Effluent EBR-3	Effluent EBR-2
	Effluent anaerobic tank
	Effluent aerobic tank
	Final effluent

Data collected included:

- Molasses Addition
- EBR Reactor Current
- EBR Reactor Voltage
- Flow rate
- ORP

Conductivity

Dissolved Oxygen

Temperature

pH

Ammonia

Nitrate

Nitrite

COD

BOD

TSS

TDS

Sulfate

Aluminum Total

Antimony Total

Arsenic Total

Barium Total

Calcium Total

Cobalt Total

Copper Total

Iron Total

Lead Total

Lithium Total

Magnesium Total

Manganese Total

Phosphorous Total

Potassium

Selenium Total

Silicon

Silver Total

Sodium

Strontium Total

Titanium Total

Zinc Total

RESULTS AND DISCUSSION

Gold Mine Results; Bench-scale

Microbial and MSM Screening Tests - Gold Mine

Results from bench-scale testing of mine waters provide guidance for pilot-scale design and operation. Fundamental bench-scale work includes microbial selection, MSM evaluation, nutrient requirements, and HRT. Results learned helped direct pilot-scale design and operation.

Figures 17 and 18 present the results obtained from microbial screening tests for the removal of nitrate from Gold mine waters. Microbes selected for screening are based on their ability to reduce nitrate to nitrogen gas. Selected microbes were grown in a sterile flask using TSB and diluted to an optical density (O.D.) of approximately 0.2, with site waters, at the beginning of the test. Nitrate concentrations and O.D. were measured and recorded at set time intervals to determine the best microbes for treatment.

In Figures 17 and 18, growth curves obtained from the absorbance OD_{600} measurements clearly show the log-phase growth portion of a classical population growth curve. Log-phase population growth is associated with high demand for growth-required resources. By selecting microbes that use nitrate as a primary electron acceptor in metabolism coupled with log-phase growth, optimum microbes for contaminant removal kinetics can be evaluated, compared, and selected for use in bench-scale testing.

Microbes selected for screening were chosen based on their capability to use nitrate as the preferred electron acceptor. Microbial growth represented by absorbance of light at 600 nm is compared to nitrate removal above. In Figure 17, microbe NJN03 removed nitrate from 200 ppm to 50 ppm, a 75% reduction in 4 hours, and exhibited the fastest removal kinetics. JWC removed nitrate to the lowest concentration of 10 ppm; a 95% reduction. The statistical significance of these results is greater than 99%. In Figure 2, WPAC, 1B, and 2B removed nitrate

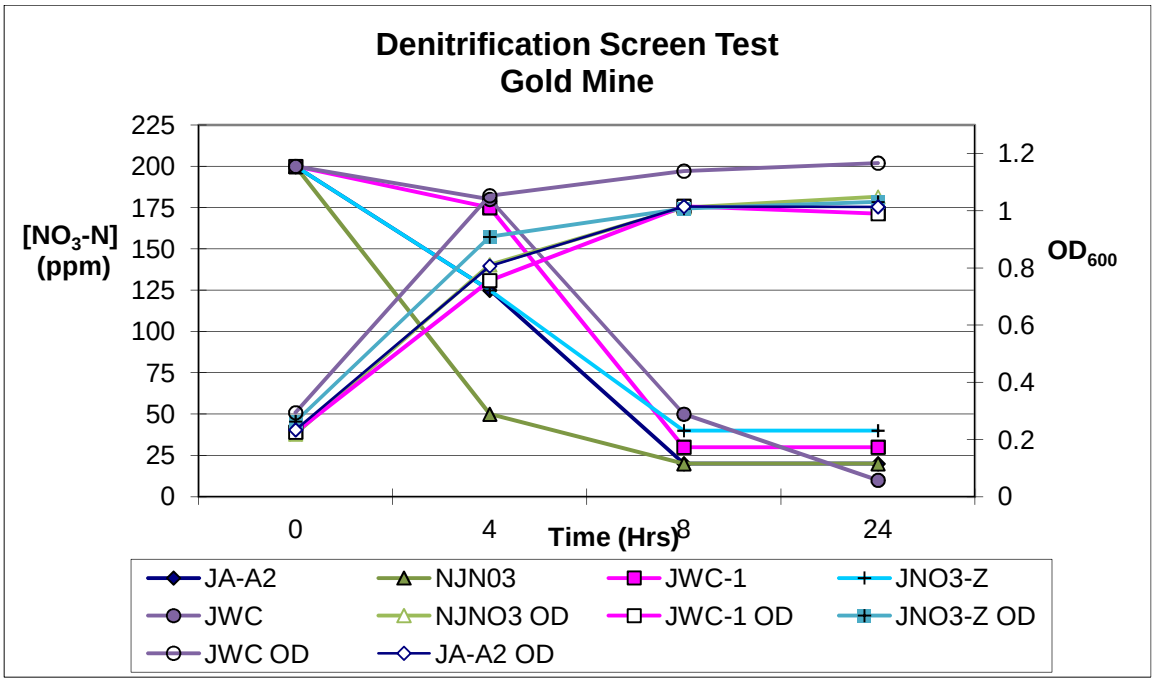

Figure 17. Nitrate removal performance and growth rate for selected microbes for the Gold mine water.

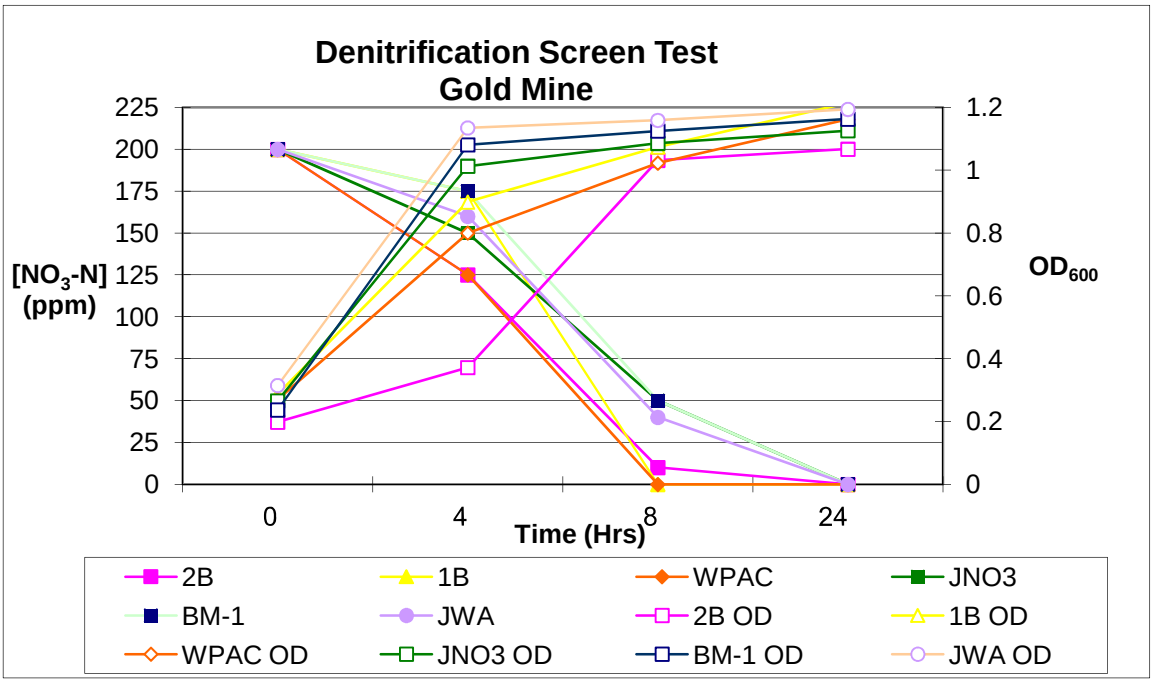

Figure 18. Nitrate removal performance and growth rate for selected microbes for Gold mine waters.

from 200 ppm to less than 10 ppm. For these particular microbes, nitrate removal performance was 25% better than the other microbes in the same test. Microbes chosen for nitrate removal from the Gold mine waters were NJNO3, JWC, WPAC, 1B, and 2B.

Results from the microbial screening test for arsenic removal from Gold mine waters are shown in Figure 19. Bacteria selected for testing were based on previous experience and their ability to reduce arsenic and a control microbe RP, which did not exhibit nitrate reduction. Samples were grown in a sterile flask with TSB nutrient. All samples were diluted to an O.D. of 0.2. Arsenic concentrations was measured and recorded at set time intervals to determine the best microbes for arsenic removal at the Gold mine.

WPAC, 1B, and 2B were chosen from the previous nitrate-screening test and used in the arsenic screen test. Bacterium BP-1 was included in the arsenic screen as it has shown the ability to reduce arsenate and arsenite in other sample waters. Results indicate that 1B and 2B have the ability to reduce arsenate as well as nitrate. There are far more bacteria with the capability to reduce nitrate than there are bacteria with the ability to reduce arsenate and arsenite. This phenomenon is in part due to the number of nitrogen-containing compounds verses the number of arsenic-containing compounds present in the biosphere as well as the energy realized from nitrate reduction versus arsenic reduction.

Bacterium 1B and 2B reduce both nitrate and arsenate/arsenite. This result has several implications. First, the energy gained from denitrification (ΔG = -2,670 kJ) is much higher than from the reduction of arsenic (ΔG = -524.3 kJ). Therefore, denitrification will proceed preferentially with these bacteria. With this understanding, EBR operation and direct electron provision can be adjusted to target nitrate removal first and arsenic removal second. Bacterial consortium development is expected to diversify within the reactor zones optimal for different contaminant transformations. Based on these results, bacterium 1B and 2B were included for arsenic removal in Gold mine waters.

In conjunction with microbial screening for contaminant removal, different microbial support materials (MSM) were screened for their effect on contaminant removal.

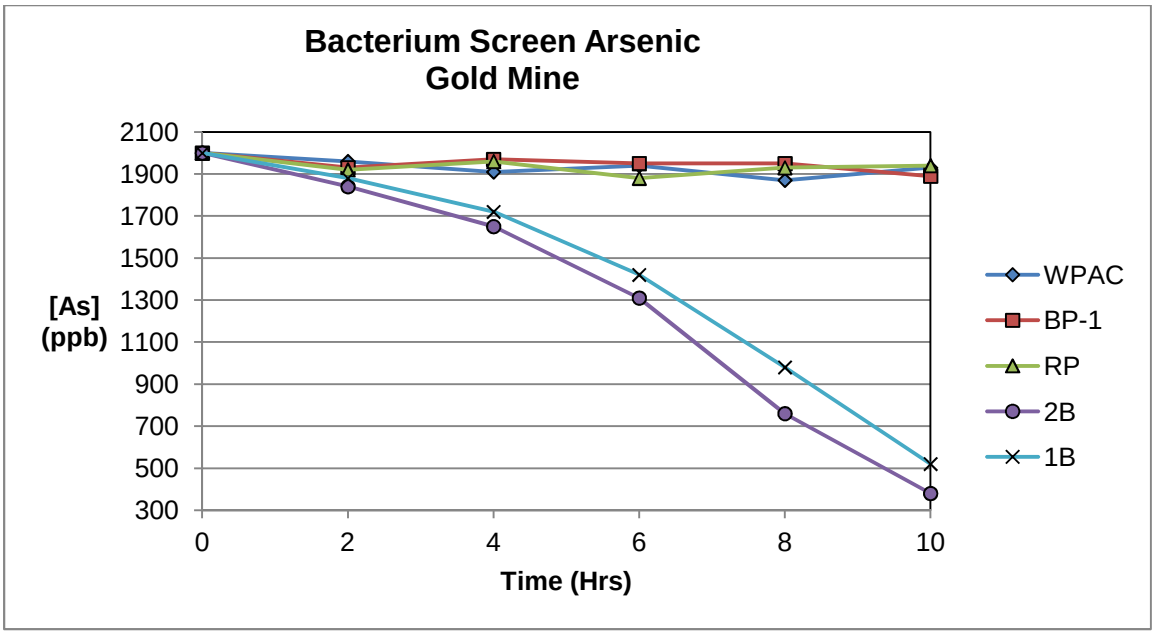

Figure 19. Arsenic removal performance for selected microbes for Gold mine waters.

Figures 20, 21, and 22 show contaminant removal results using different MSM inoculated with the same volume of a microbial population. Figures 20 and 21 are the results obtained from MSM screening for nitrate removal. Figure 22 provides the results obtained from MSM screening and arsenic removal.

In Figure 20, GAC and pumice outperformed scoria, which is a solid rock with no pore structure. Results indicate that porous structures improve nitrate removal by up to 80% over MSM with no pores. Further analysis indicates that acid washed MSM outperforms simple washing with de-ionized water alone. The statistical significance of this result is greater than 95%.

Figure 21 illustrates the difference between pelletized AC, GAC, and pumice rock. GAC pore structure is significantly smaller than pumice and therefore increases surface area for microbial attachment. Overall for denitrification, pelletized GAC provided the best removal results with 100% removal after 6 hours, followed by GAC with 100% removal after 7 hours, and finally pumice with total nitrate removal after 8 hours. However, the difference in removal kinetics and the cost, over $1.00/lb more for GAC and pelletized AC, did not warrant the added benefit. Removal of nitrate by absorption to GAC was not tested as the U.S. EPA's analysis indicates nitrate does not absorb to GAC; no sorption test was performed (104).

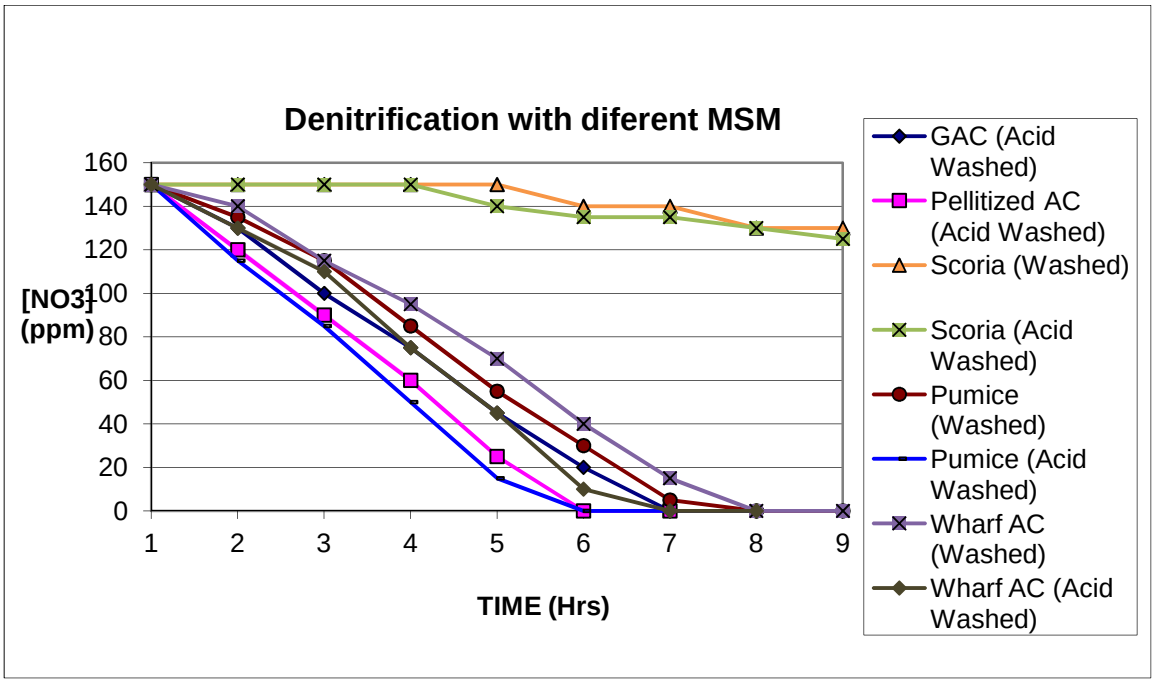

Figure 20. Nitrate removal with different MSM, Gold mine water.

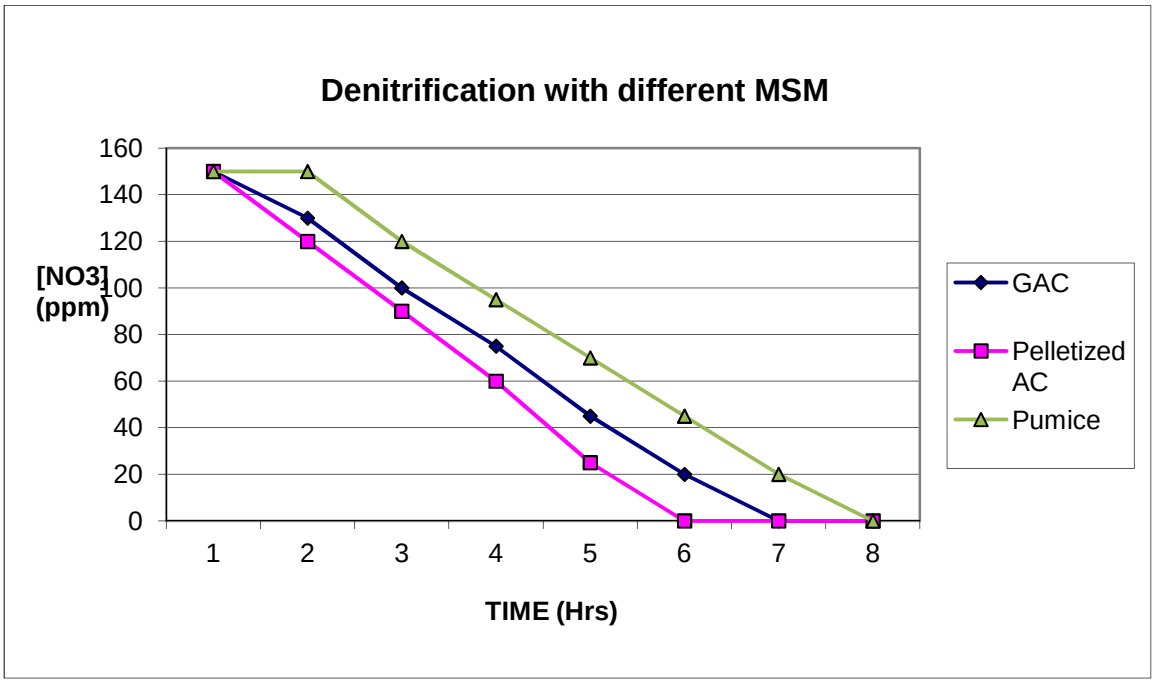

Figure 21. Nitrate removal with different MSM, Gold mine water.

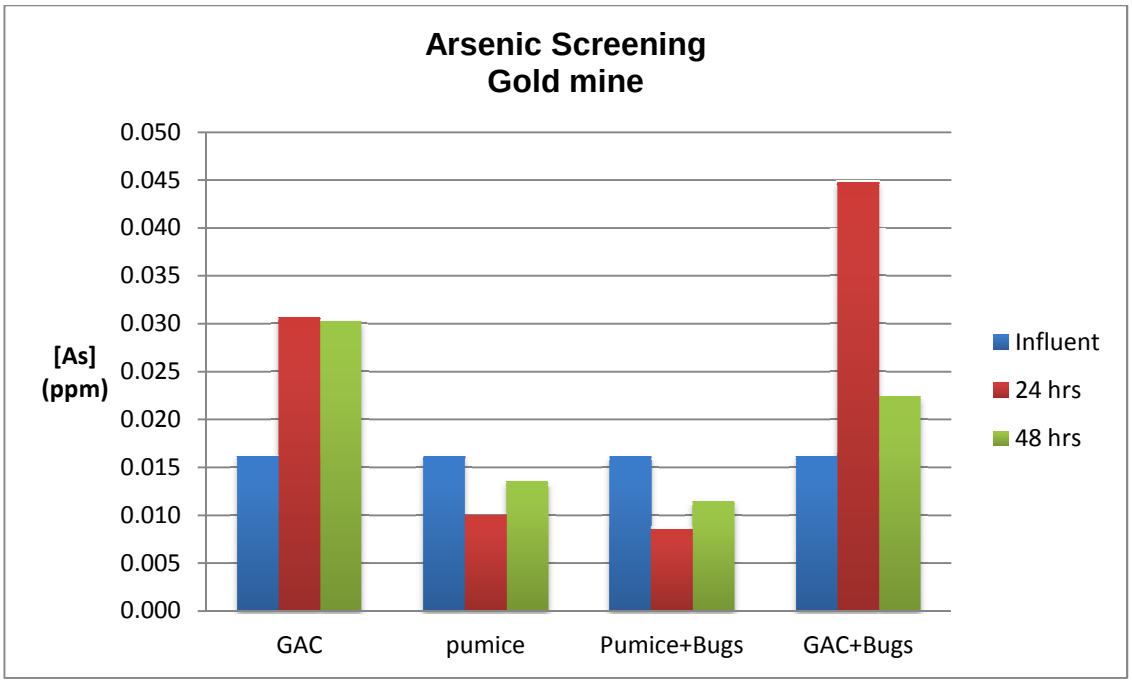

Figure 22. Arsenic removal with different MSM, Gold mine water.

In Figure 22 GAC, pumice, inoculated pumice, and inoculated GAC were tested to examine absorption characteristics and removal kinetics of arsenic. Results indicate GAC increased overall arsenic concentration from 0.016 ppm to 0.030 ppm over 48 hours; arsenic is leaching from not absorbing to this particular GAC. Additionally, inoculated GAC showed an increase from 0.016 ppm to 0.022 ppm in final arsenic concentration after 48 hours. This effect was unexpected; it was later learned, from the manufacturer that source material for GAC includes walnut and coconut shells and based on the geographic region and historical industrial activities, arsenic may be present in GAC source materials.

Increased arsenic concentration was unexpected as some sorption of arsenic by GAC was expected. Research shows that absorption of arsenic by untreated GAC is highly dependent on speciation and pH and is fairly ineffective for arsenic removal (105). Absorption characteristics were examined with pumice as well and results indicate initial arsenic concentration of 0.016 ppm and a final arsenic concentration of 0.014 ppm. Overall results indicate inoculated pumice provide the best arsenic removal results with 32% removal in 48 hours. Based on this result and the cost

of GAC; pumice was used as the main constituent for the MSM in the Gold mine EBR reactors. GAC was examined as an additional conductive surface material with the electrodes located at the midpoint of the GAC layer; the rest of the bioreactor MSM was filled with pumice. No affect was observed with the use of GAC as an addition conductive surface material in these tests.

Bench-scale EBR Tests – Gold Mine

Two identical bench-scale reactors were constructed and operated continuously as described in the materials and methods section to compare bioreactors operated with and without directly applied electrons. All parameters, other than the direct application of electrons, were held as equal as possible, including microbial inoculum, HRT, and nutrient components and amounts. Bench-scale bioreactors were built identically as described in the materials and methods section.

Figures 23, 24, and 25 provide the results of about 2 months of EBR operation for the treatment of nitrate and arsenic in Gold mine waters.

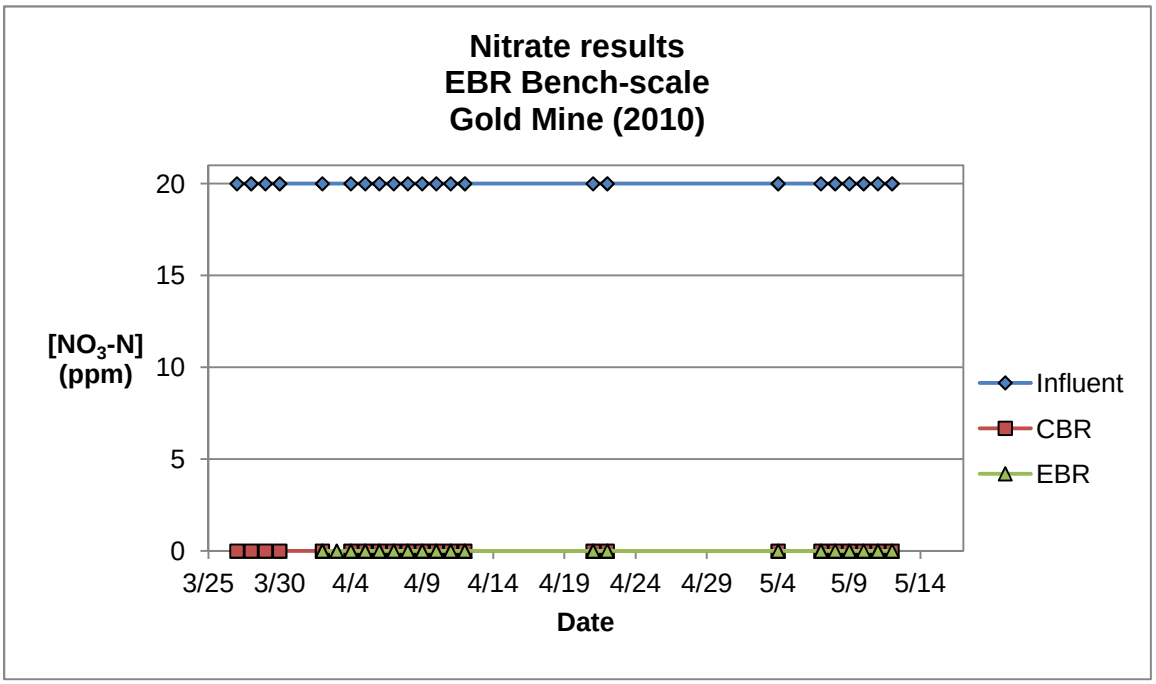

Figure 23. Bench-scale EBR vs. a conventional bioreactor (CBR) for nitrate removal from Gold mine waters.

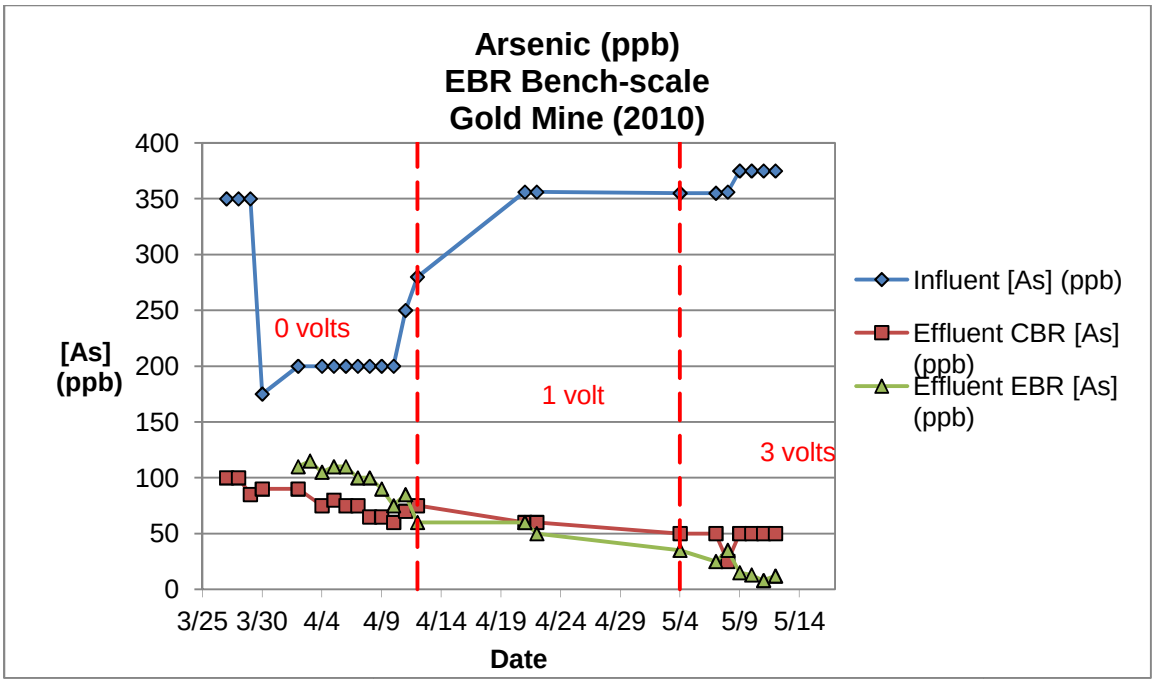

Figure 24. EBR vs. CBR arsenic concentration with EBR applied voltage.

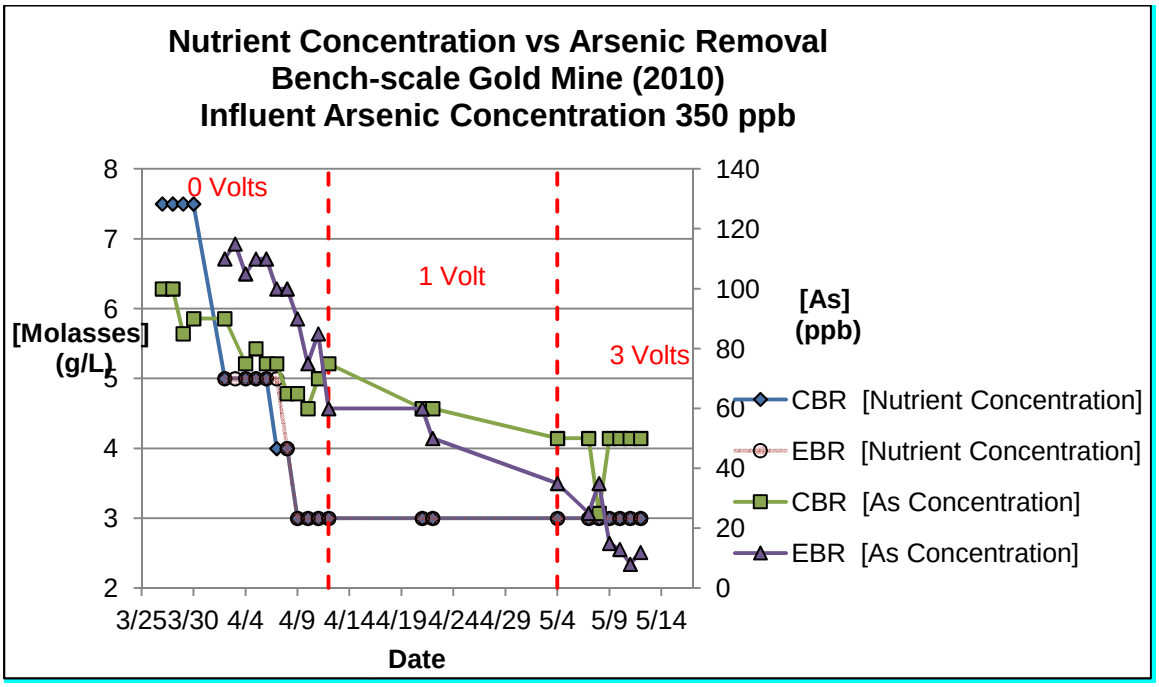

Figure 25. EBR vs. CBR effluent arsenic concentration with nutrient addition using Gold mine waters.

Figure 23 shows the results for influent and effluent nitrate-N concentrations from EBR and CBR bioreactors. Nitrate removal over a 24-hour period was the same for both bioreactor types. These results are not unexpected, as nitrate-screening tests indicated complete nitrate removal within 8 hours as shown in Figures 17, 18, 20, and 21. It is also important to note that screening tests did not include directly applied electrons. Direct electron addition has been demonstrated to significantly increase nitrate removal kinetics in other documents describing EBR operation.

Figure 24 illustrates the advantage of direct electron addition in the EBR system. In this test, a CBR and an EBR were run side by side keeping all aspects identical other than the addition of an applied voltage. In these tests, direct electron addition was between 4.6×10^{15} electrons/sec and 13.8×10^{15} electrons/sec at both 1 and 3 volts. The voltage potential in these experiments indicated the energy of the electrons provided to the system.

Influent arsenic concentration during the test averaged 280 ppb. The EBR was started a week later than the CBR due to logistical issues. Both reactors were given a 2-week stabilization period for the establishment of the microbial biofilm in the Gold mine waters. Between 4/12 and 5/4, a 1-volt potential was applied to the EBR reactor and arsenic concentrations for both effluents were measured and recorded. During this period, the CBR average arsenic effluent concentration was 60 ppb and the EBR average arsenic effluent concentration was 50 ppb. On 5/4, voltage to the EBR was increased from 1 volt to 3 volts until the end of the test on 5/12. EBR effluent arsenic averaged 13 ppb while CBR arsenic effluent averaged 50 ppb. Calculating a value for the z test at three volts provided a value of $z = 4.83$. This value passes the test for a 99% confidence interval. Based on these and other bench test results, a pilot-scale test at the Gold Mine was requested.

Figure 25 present's results from the bench-scale testing of the Gold mine waters for arsenic removal with respect to nutrient concentration addition. Both EBR and CBR nutrient concentrations of 3 g/L were held equal from 4/8 to the end of the test on 5/12. Figure 25 shows an approximate difference in final arsenic concentration of 40 ppb between the CBR and the EBR over the final 10 days of the test. CBR effluent arsenic concentration averaged 50 ppb while the

EBR final arsenic effluent averaged 13 ppb. Influent arsenic concentrations averaged 350 ppb during the final period of testing.

Gold Mine Results; Pilot-scale

Validation of the EBR process at pilot-scale examines EBR system performance using larger water flows from 1 to 4 liters and site environmental conditions, including influent arsenic fluctuation. This testing defines the operational boundaries for a full-scale EBR process as well as provides information on potential operational issues. The effects of HRT, nutrient concentrations, and temperature on removal of nitrate and arsenic were examined. The pilot-scale system described in the materials and methods was set up on-site at the gold mine and the data collected are graphed and discussed in Figures 26-28.

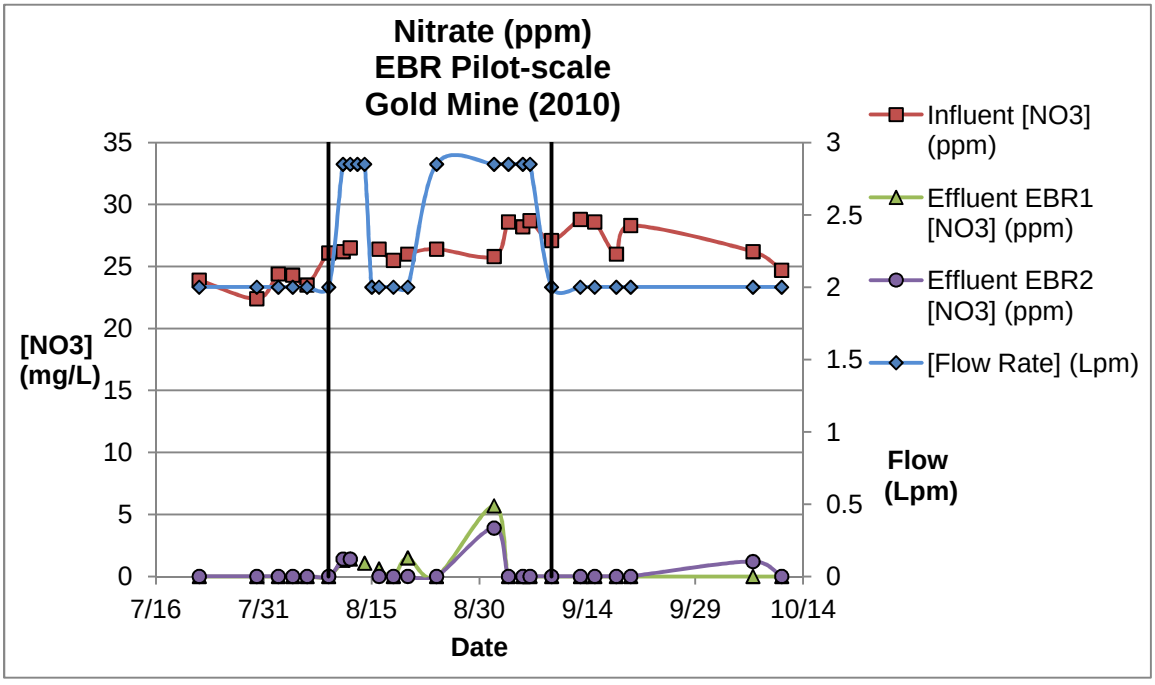

Figure 26. Nitrate-N removal and flow rate for EBR pilot-scale, Gold mine.

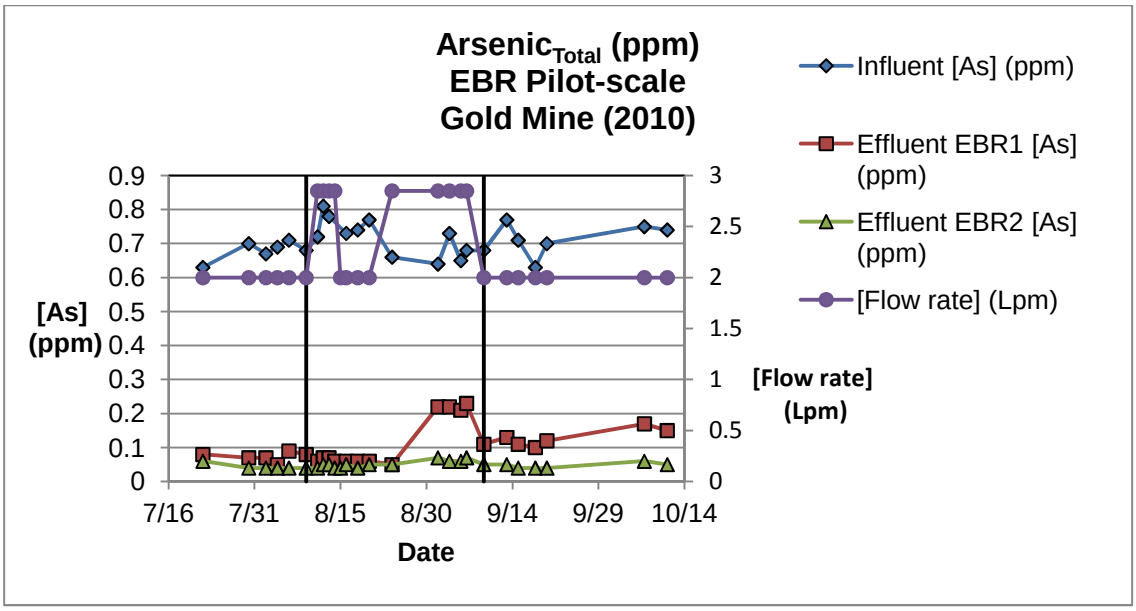

Figure 27. Arsenic removal results with flow rate for EBR pilot-scale, Gold mine

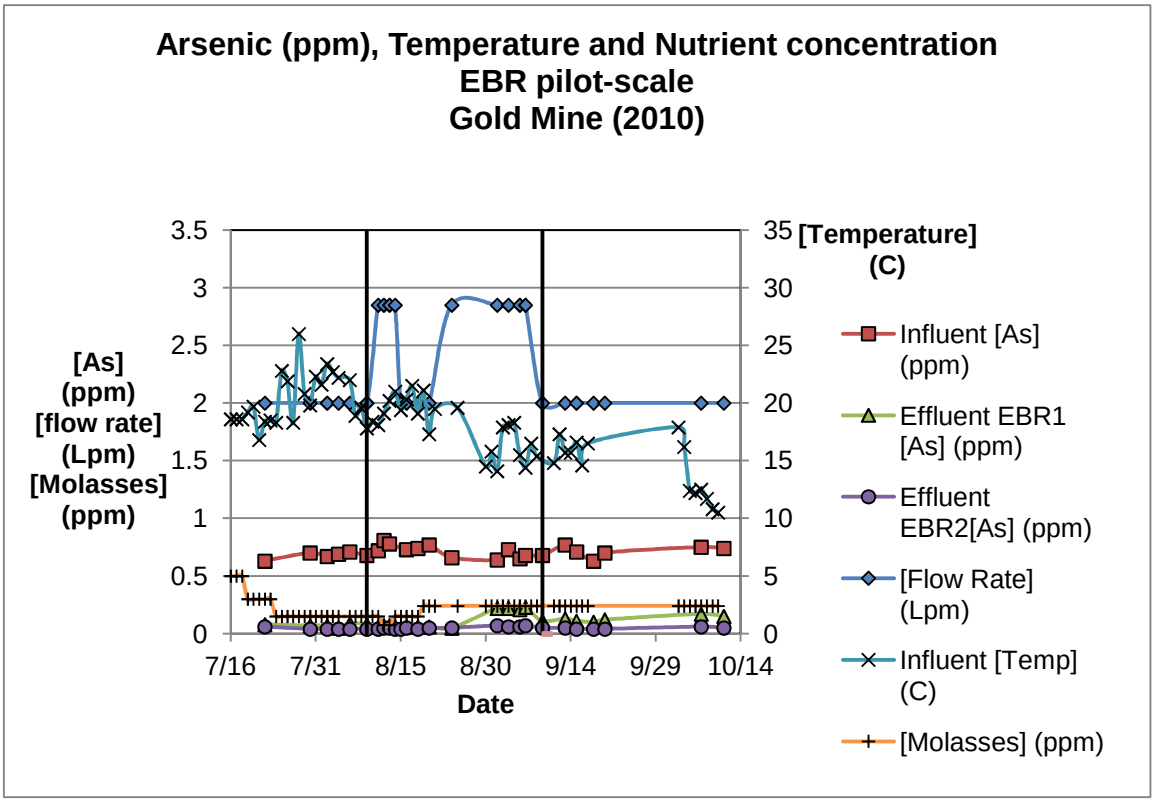

Figure 28. Arsenic removal results and water temperature, EBR pilot-scale, Gold mine.

Figure 26 are the data collected from the Gold Mine pilot test for nitrate. EBR pilot testing was divided into three periods: period one ran from 7/22 to 8/9 and consisted of validation of the EBR systems removal performance at the design flow rate of 2 Lpm; period two ran from 8/10 to 9/8 and consisted of system stressing with respect to flow rates of 2.85 Lpm; period three ran from 9/9 to 10/11 and was marked by a 7.4^0 C drop in influent water temperature from 17.9^0 C to 10.5^0 C. Table 12 is a summary of the results found in Figure 26.

Complete nitrate removal was typically observed in the discharge of EBR 1 and 2. Flow rates were increased twice during pilot testing from 8/11 to 8/14 and from 8/21 to 9/5 from 2 lpm to 2.85 lpm. During flow rate increase on 8/11, nitrate increased from an average of 0 ppm to an average of 1.1 ppm; discharge criteria were 10 ppm.

Temperature during this period dropped from 17.9^0 C to 10.5^0 C. The last nitrate test for the Gold mine EBR pilot measured was on 10/11 and final effluent nitrate for both EBR 1 and 2 was 0 ppm. Total average nitrate influent concentration for the entire test was 27 ppm and final average nitrate effluent for EBR 1 was 0.241 ppm and for EBR 2 was 0.07 ppm, well below the nitrate effluent target of 10 ppm.

Figure 27 is the arsenic removal data collected with respect to flow rate. Table 13 is a summary of the results found in Figure 27. The black lines on the graph correspond to three periods, including validation, flow rate stressing, and temperature stressing, respectively.

Influent arsenic concentrations averaged 0.71 ppm for the entire pilot test. EBR-1 and EBR-2 had a 15-hour retention time at a flow rate of 2 Lpm and 10.3-hour retention time at 2.85 Lpm. EBR-1 effluent arsenic concentrations averaged 0.1 ppm or 66% removal and EBR-2

Table 12. Flow rate, average nitrate influent and effluent, EBR pilot Gold mine.

Date	**Average flow rate (Lpm)**	**Average influent NO_3-N (ppm)**	**Average effluent EBR-1 NO_3-N (ppm)**	**Average effluent EBR-2 NO_3-N (ppm)**
7/22-8/9	2	24.1	0	0
8/10-9/8	2.85	26.8	1.1	0.7
9/9-10/11	2	27.1	0	0.2

Table 13. Flow rate, average arsenic influent and effluent, EBR pilot Gold mine.

Date	Average flow rate (Lpm)	Average influent total As (ppm)	Average effluent EBR-1 total As (ppm)	Average effluent EBR-2 total As (ppm)
7/22-8/9	2	0.68	0.07	0.043
8/10-9/8	2.85	0.72	0.11	0.051
9/9-10/11	2	0.72	0.12	0.047

averaged 0.047 ppm or 94.4% removal over the entire test period. An increase in arsenic effluent from 0.05 ppm to 0.22 ppm in EBR-1 occurred on 8/30 corresponding to an increase in flow rate from 2 Lpm to 2.85 Lpm. During the same period, EBR-2 effluent arsenic increased from 0.043 to 0.051 ppm during HRT stressing. On 9/9, flow rate was adjusted back to 2 Lpm. Between 10/3 and 10/11, EBR pilot influent water temperature dropped from 17.9^0 C to 10.5^0 C. From 10/3 to 10/7, water temperature dropped from 17.9^0 C to 12.2^0 C. During the same period, EBR-1 effluent arsenic increased from 0.12 to 0.17 ppm and EBR-2 effluent arsenic increased from 0.04 to 0.06 ppm. EBR arsenic removal performance did improve from 10/7 to 10/11 as temperature continued to drop from 12.2^0 C to 10.5^0 C. EBR-1 effluent arsenic dropped from 0.17 to 0.15 ppm and EBR-2 effluent arsenic dropped from 0.06 to 0.05. The data indicate that even as arsenic concentrations and temperature fluctuated, the microbes were able to acclimate and maintain a uniform final arsenic effluent concentration. The data indicate that lower temperatures did not impact final arsenic concentrations.

EBR quality control for the water analysis is presented in Table 14; quality control samples were splits or dilutions of effluent water samples for the Gold mine pilot testing. A review of quality control data produced several unexpected trends. Influent duplicate water sample results on 8/12 returned values of 0.71 and 0.68 ppm. EPA guidelines for quality control indicate duplicate samples within ±10% of each other are acceptable (106); influent sample differences were less than 4.7%. With respect to EBRs 1 and 2, 10-fold diluted samples showed no difference in final arsenic concentration from undiluted samples. Based on these results, a sample from EBR-2 was prepared and sent to an alternate lab along with a paired sample to the

Table 14. Quality control tests for arsenic, EBR pilot Gold mine.

Date	Sample	Result	Expected result
8/12/2010	Influent	0.71 ppm	
8/12/2010	Influent (paired sample)	0.69 ppm	0.71 ppm
8/12/2010	EBR-1 effluent	0.073 ppm	
8/12/2010	EBR-1 effluent (1:2 dilution)	0.068	0.0365 ppm
8/12/2010	EBR-2 effluent	0.046 ppm	
8/12/2010	EBR-2 effluent (1:2 dilution)	0.051 ppm	0.023 ppm
8/12/2010	EBR-2 effluent (Alternate certified lab results)	0.010 ppm	0.046
9/5/2010	Influent	0.64 ppm	
9/5/2010	EBR-1 effluent	0.136 ppm	
9/5/2010	EBR-1 effluent (1:2 dilution)	0.127 ppm	0.068 ppm
9/5/2010	EBR-2 effluent	0.051 ppm	
9/5/2010	EBR-2 effluent (1:2 dilution)	0.055 ppm	0.025 ppm
9/8/2010	Influent	0.68	
9/8/2010	EBR-1 effluent	0.144	
9/8/2010	EBR-1 effluent (1:2 dilution)	0.142	0.072 ppm
9/8/2010	EBR-2 effluent	0.061	
9/8/2010	EBR-2 effluent (1:2 dilution)	0.059	0.031 ppm

primary lab. The result returned from the alternate lab indicated a final effluent concentration of 0.010 ppm while primary lab results indicated a value of 0.046. In fact, in reviewing the primary lab data results, it appears that analysis of arsenic concentrations below 0.046 were unachievable. Based on the quality control analysis, EBR effluent arsenic concentration results reported herein are likely significantly lower than the actual effluent arsenic concentrations reported.

Figure 28 presents the arsenic removal data with temperature, flow rate, and nutrient concentration data included for the EBR Pilot-scale Gold Mine test. Figure 28 is important as reaction thermodynamics are influenced by temperature as represented by “T” in the Gibbs free energy equation $\Delta G = \Delta H - T\Delta S$. The influence of temperature on arsenic removal performance is best illustrated in Figure 28 by the increase in EBR arsenic effluent concentrations on 10/9.

EBR-1 arsenic effluent increased from 0.12 on 9/20 to 0.17 on 10/7 while influent water temperature dropped from 17.9^0 C to 12.2^0 C. EBR-1 arsenic removal performance did, however, improve from 10/7 to the end of test on 10/11 and arsenic effluent decreased from 0.17 ppm to 0.15 ppm even as temperature dropped from 12.2^0 C to 10.5^0 C as the microbes acclimated to the lower temperatures. In the same time period, EBR-2 arsenic effluent concentrations increased from 0.04 ppm to 0.06 over the same temperature drop. Arsenic removal performance in EBR-2 improved from 10/7 to 10/11 from 0.06 ppm to 0.05 ppm. Figure 29 is the ORP measured during the Gold mine pilot test. Table 15 is a summary of the results in Figure 29.

ORP values in EBR-1 ranged from a low of -351 mv to a high of -80 mv. ORP values for EBR-2 ranged from a low of -301 mv to a high of -124 mv. Optimum ORP values for arsenic reduction are between -150 mv and -200 mv as shown in the Eh-pH diagram in Figure 2. The EBR process utilizes electrons for ORP control and stability. Analysis of the standard deviation of ORP during different HRT's and decreasing temperatures is required for full-scale system design.

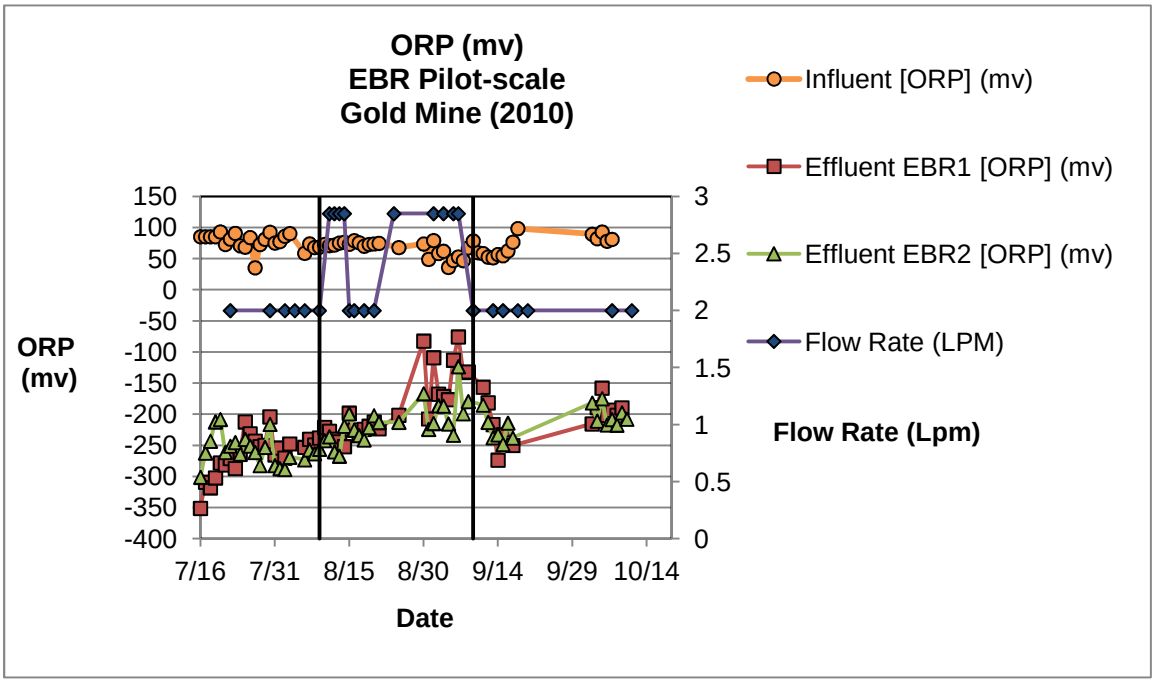

Figure 29. ORP data and flow rate from EBR pilot-scale, Gold mine.

Table 15. Flow rate and average ORP, EBR pilot, Gold mine.

Date	Flow rate (Lpm)	EBR-1 Average ORP (mv)	EBR-1 Standard Deviation	EBR-2 Average ORP (mv)	EBR-2 Standard Deviation	Standard Deviation/average ORP (%) EBR-1/EBR-2
7/15-8/10	2	-264.3	35.5	-258.6	39.6	13.4/15.3
8/11-8/14	2.85	-240.8	10.4	-245.5	22.1	4.3/9.0
8/24-9/6	2.85	-144.8	50.4	-194.7	34.4	34.8/17.7
9/7-10/10	2	-197.7	24.3	-209.4	21.7	12.3/10.3
10/3-10/10	2	-192.5	16.4	-201.7	16.0	8.5/7.9

The EBR pilot system was run under two different flow rates: 2 Lpm and 2.85 Lpm. Influent ORP averaged 72.3 mv and the standard deviation was 14 mv for the entire test. From 7/15 to 8/10 and from 9/7 to 10/10, the flow rate was held constant at 2 Lpm. From 7/15 to 8/10, EBR-1 effluent ORP averaged -264.3 mv and the standard deviation was 35.54 mv. During the same time period, the average ORP for EBR-2 was -258.6 mv and the standard deviation was 39.6 mv. From 9/7 to 10/10, the flow rate was again held constant at 2 Lpm. The average ORP for EBR-1 was -197.7 mv and the standard deviation was 24.3 mv. EBR-2 ORP averaged -209.4 mv and the standard deviation was 21.7 mv for the same period.

Examination of the relationship between average ORP with respect to standard deviation between the two time periods, at which flow rate was 2 Lpm, shows a percent of variance of 13.4% versus 12.3% for EBR-1 and 15.3% and 10.3% for EBR-2 for the respective time periods. On 8/11 to 8/14 and from 8/24 to 9/6, flow rate was increased to 2.85 Lpm. The average ORP for EBR-1 from 8/11 to 8/14 was -240.8 mv and the standard deviation was 10.44 mv. From 8/24 to 9/6, the average ORP was -144.8 mv and the standard deviation was 50.38 mv. For EBR-2, average ORP for the respective time periods was -245.5 mv and -194.7 mv and the standard deviation was 22.10 mv and 34.43 mv. Calculating percent of variance for EBR-1 at a flow rate of 2.85 Lpm for each time period yields 4.3% and 34.8%. EBR-2 variance for the higher flow rate was 9% and 17.7%. Average ORP and standard deviation from 10/3 to 10/10, corresponding to a

temperature drop of 7.4 C, was -192.5 mv and -201.7 mv and 16.4 mv and 16.0 mv for EBR-1 and EBR-2, respectively. The variance for EBR-1 and EBR-2 was 8.5 and 7.9.

Analysis of the ORP statistics provides insight into the effect of electron addition, temperature and nutrient concentration in the EBR reactors. From 7/15 to 8/10 and from 9/7 to 10/10, the EBR system was run at the design flow rate of 2 Lpm and average ORP values ranged the lowest. Nutrient effects are evident in the average ORP values between the two periods excluding the temperature drop period. Nutrient addition in the first period averaged 2.75 g/L and 2.4 g/L in the third period. ORP values increased from -263 mv to -197 as average nutrient concentration was reduced. The difference in ORP variance between the two periods was about 1.1 for EBR-1 and 5 for EBR-2.

The analysis for flow rates corresponding to 2.85 Lpm is tempered somewhat by operational issues. On 8/14, site power was interrupted and the EBR system was nonfunctional until 8/16. To minimize the influence of power loss on the data, the EBR system was restarted at a flow rate of 2 Lpm to establish baseline performance. On 8/24, flow rate was again increased to 2.85 Lpm. In an effort to provide transparency, the data analyzed from 8/10 to 8/14 are included; however, due to the low number of observations for this period, statistical analysis results have been discounted. Average ORP values at the higher flow rate, -144.8 mv and -194.7 mv, and standard deviations, 50.4 and 34.4, for EBR-1 and 2 were the highest observed during pilot testing.

The effect of temperature on ORP was the last period analyzed. While keeping flow rate and nutrient constant, the effect of temperature can be seen in the statistics. EBR-1 saw a 5.2 mv increase and EBR-2 saw a 7.7 mv increase in ORP. However, analysis of variance during this period produced the smallest values of 8.5 and 7.9 for EBR-1 and 2, respectively. The addition of electrons on ORP stability is evident in the results from the Gold mine EBR pilot test. The ability to target specific ORP values to coincide with the thermodynamics of target contaminates provides reactor stability and reliability to meet discharge targets.

Base Metal Mine Results; Bench-scale

Microbial and MSM Screening Tests - Base Metal Mine

Microbial screening in Base Metal mine waters were focused on selenium reduction. These screening tests differed from those for arsenic and nitrate in that the microbial screening for selenium reduction is based on subjective evaluation of selenium precipitates formed and sample turbidity. Three different conditions were tested.

- Augmented mine water spiked with 25 ppm selenium
- Augmented mine water spiked with 2 ppm selenium
- Unaugmented mine water

All microbial screening tests were completed in duplicate and the results were evaluated over the three test conditions of 1) Quantity of precipitates, 2) Quality of precipitates, and 3) Sample turbidity. Quantity implies the amount of precipitate formed in the test tube and a rank of three corresponds to the large quantity observed. Quality refers to the nature of the precipitate and weather it is amorphous or distinct; a rank of three relates to a distinct well-formed selenium precipitate in the bottom of the test tube. Turbidity implies inefficient settling of the precipitates, a rank of three corresponds to clear solution inside the test tube. Broad spectrums of microbial isolates were screened for this test, as influent water chemistry components include residual flotation reagents and a high variability in selenium concentration. Figures 30 thru 35 represent the results from microbial screening tests from the Base Metal mine waters.

Figures 30 and 31 represent each microbe tested with quantity and quality of the precipitates and the turbidity of the solution ranked on a scale of one to three, with three being best. Figures 30 and 31 are duplicate tests and overall selection of best performing microbes is based on the average performance between the two tests. $As^{1}B$, SF005a, $Se24^{-1}$ A and E, Se2u A, B, and E, and Se2uD iso performed well over both tests and received ranks of two or better for at least two of the categories in both tests.

Figures 32 and 33 are results from the screening of microbes using Base Metal mine water spiked with 2 mg/L of selenium. Again $As^{1}B$, SF005a performed well receiving high marks for at least two categories over both tests. $Se24^{-1}$ A and E and Se2u A, B, and D again did well

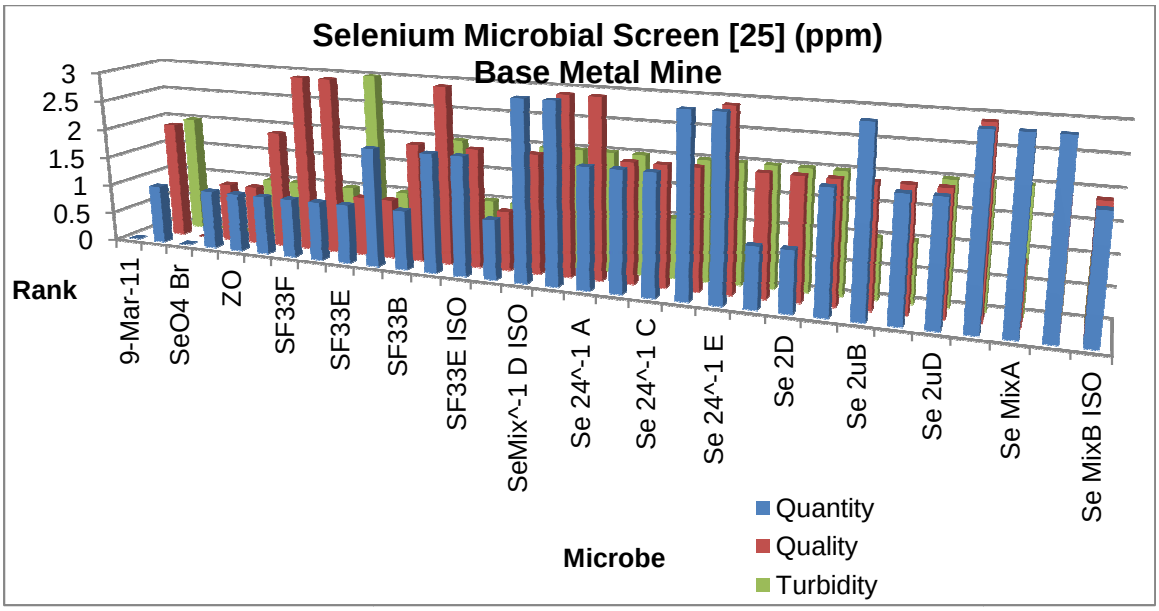

Figure 30. Microbial screen test for Base Metal mine water 3/9/2011.

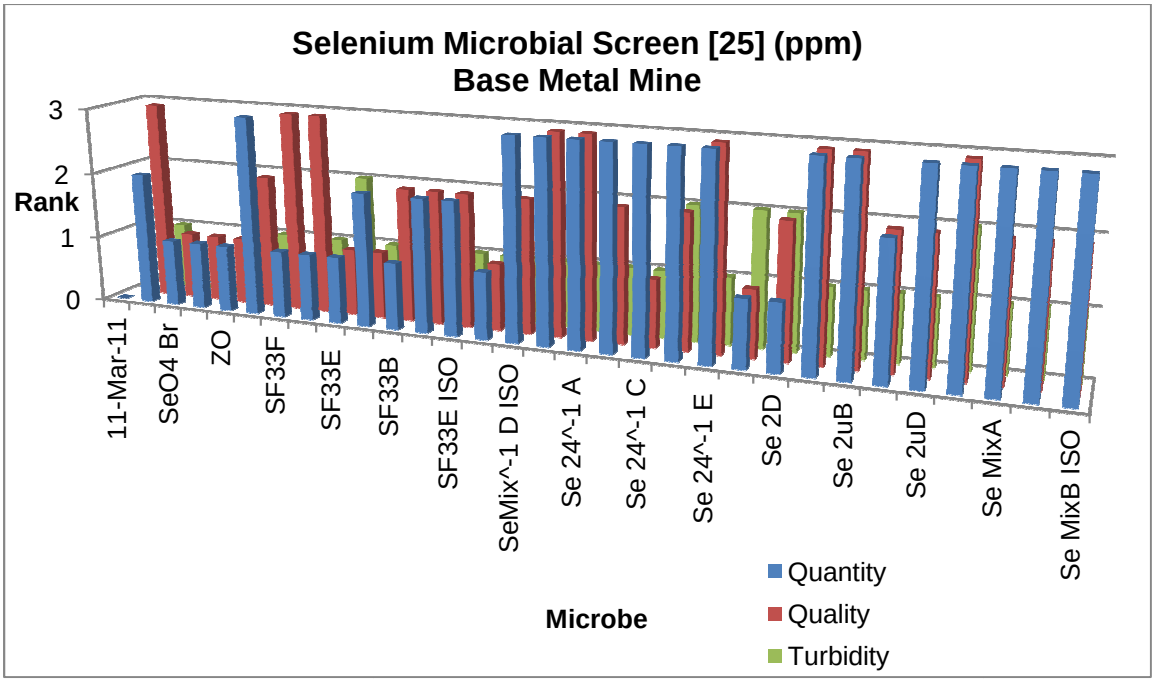

Figure 31. Microbial screen test for Base Metal mine water 3/11/2011.

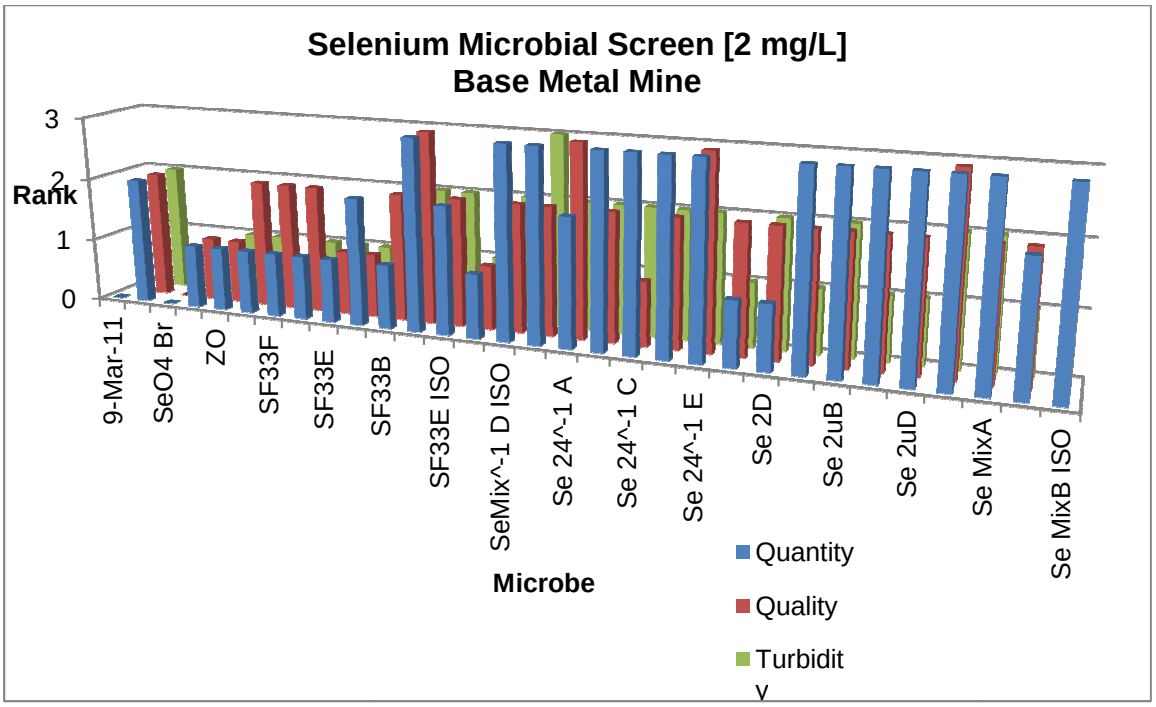

Figure 32. Microbial screen test for Base Metal mine water 3/9/2011.

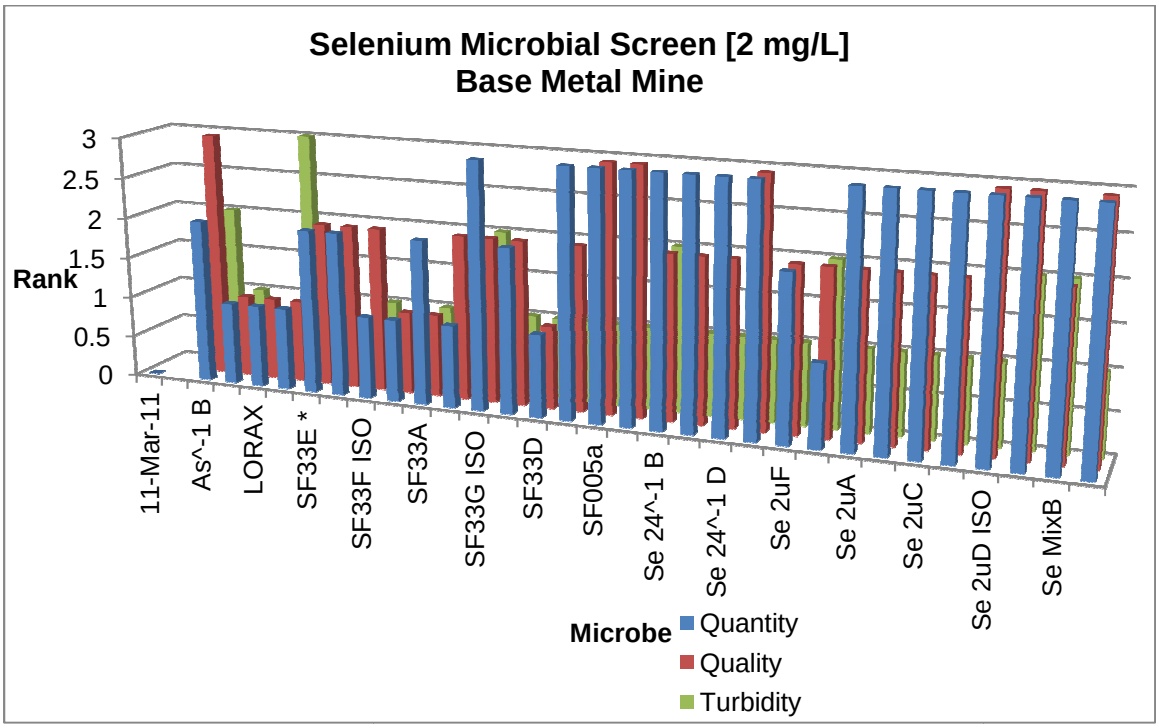

Figure 33. Microbial screen test for Base Metal mine water 3/11/2011.

over both tests with high marks in all three categories. Se Mix A and B's performance was noted; however, results were mixed over the two tests.

Figures 34 and 35 represent the results for selenium removal from microbial screening tests for the unaugmented Base Metal mine water. The water chemistry in this test are the conditions most likely to be encountered during pilot-scale testing and analysis of these results carry the most weight of the three test conditions described by Figures 30 thru 35. Analysis of these results provide clear guidance in choosing which microbes should be selected and examined for use at this particular mine site. Microbes tested include As^{-1}B, SF33G ISO, SF005a, Se 24^{-1} A, B,C, D, E, and Se2uD ISO. Review of results from the three different test conditions reveals the best microbes. As $^{-1}$B, SF005a, Se24^{-1} A, and Se24^{-1} E under all test conditions provided the most stable performance in all three categories.

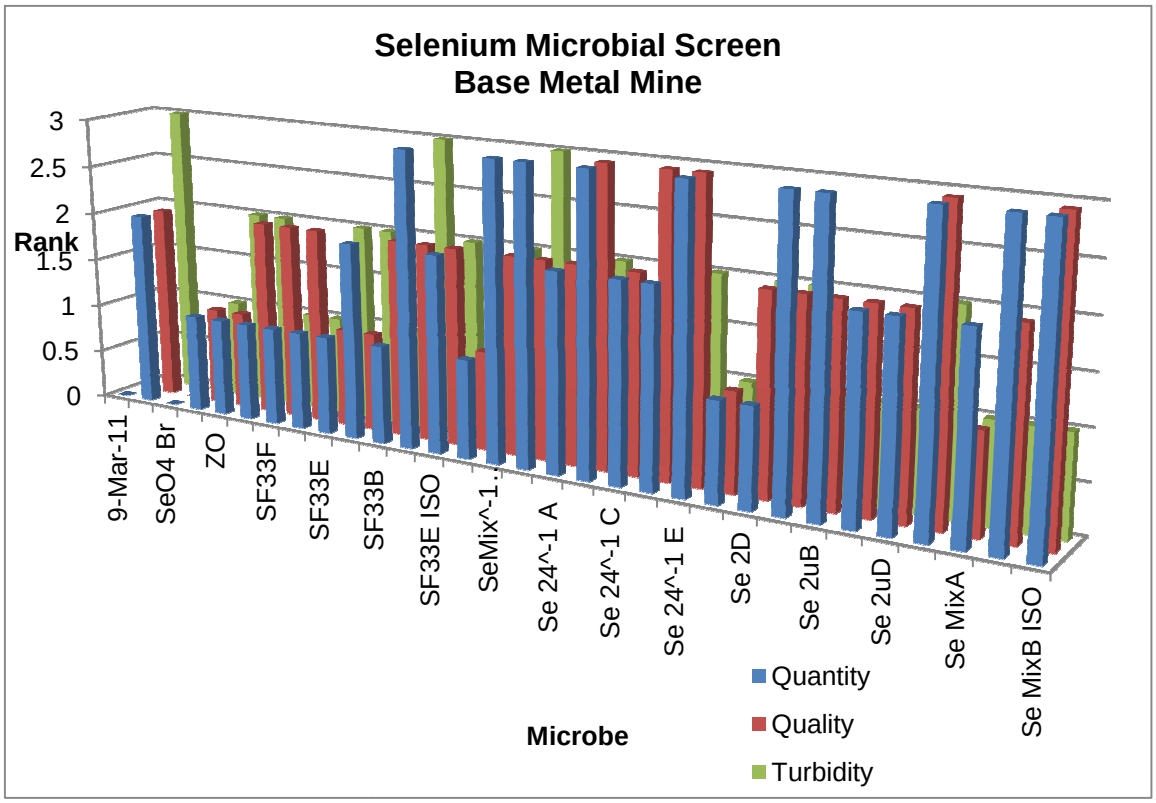

Figure 34. Microbial screen test for Base Metal mine water 3/9/2011.

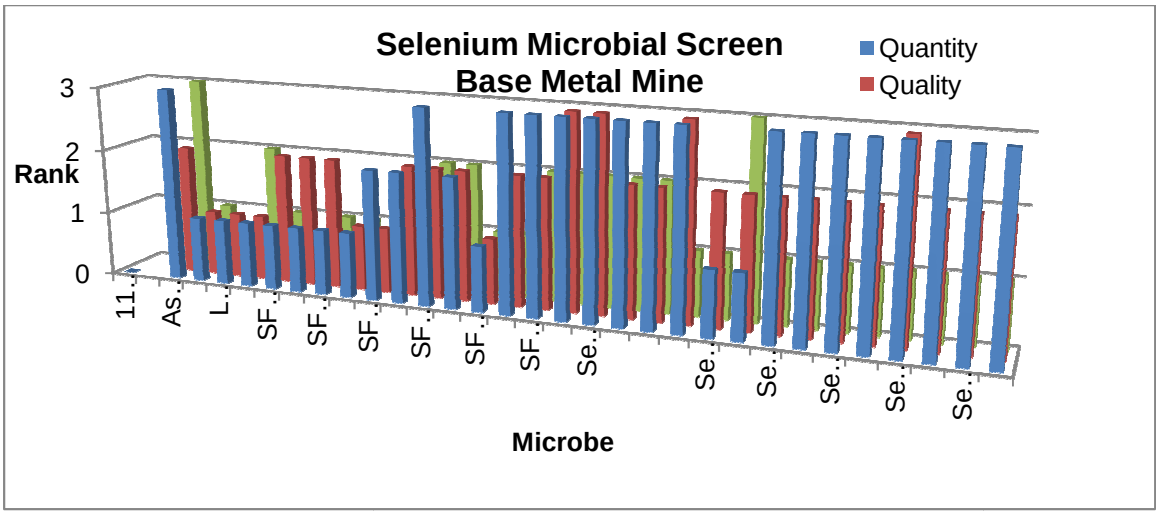

Figure 35. Microbial screen test for Base Metal mine waters 3/11/2011.

In conjunction with the microbial screenings was the evaluation of different MSM and their effect on overall selenium removal. Figure 36 shows results obtained from the MSM screen. Four different MSM tests were run on the Base Metal mine waters. The first test included only GAC to examine absorption characteristics with selenium. Results show that no absorption occurred and in fact, selenium increased from 3.17 ppm to 3.38 ppm; this was due to the selenium content of the GAC tested. The statistical significance of the increased selenium concentration passes a 99% confidence level. A microbial consortium was developed based on the results of microbial screening in the previous section. Pumice and GAC were inoculated with the selected microbes and a separate flask was examined with only microbes.

One liter of Base Metal mine water was then added to each flask. Water samples were collected initially and at 24 and 48 hours and analyzed for selenium. Initial selenium concentrations in the three flasks were 3.17 ppm. After 24 hours, the flask containing only microbes had a selenium concentration of 0.74 ppm and the flask containing pumice and microbes had a concentration of 0.79 ppm. Statistical analysis shows no difference between these two results. However, the flask containing GAC and microbes had a selenium concentration of 0.21 ppm. GAC and microbes had a 94% removal of selenium while the pumice and microbes and the microbes alone only removed 75% of selenium; GAC was selected as the best MSM for the Base Metal mine water EBR tests.

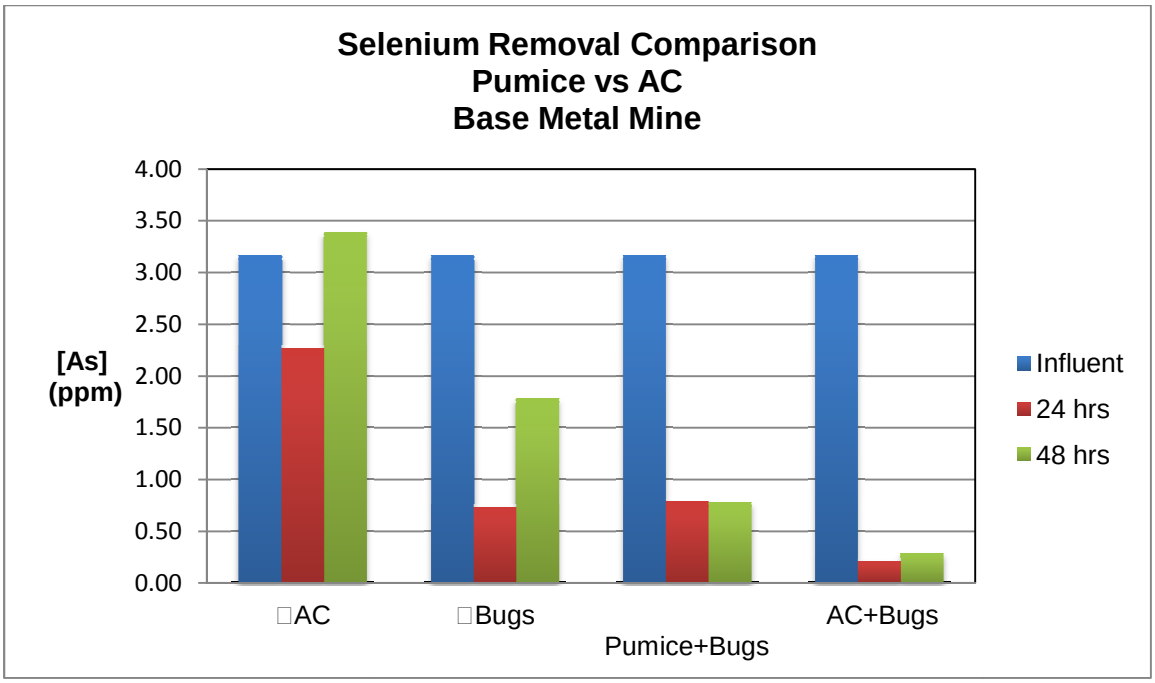

Figure 36. Selenium removal performance for different MSM.

Bench-scale EBR Tests – Base Metals Mine

Bench-scale testing of Base Metal mine waters occurred over 6 months. The Base Metal mine is an underground mine that uses flotation and concentration in the separation of lead, zinc, copper, silver, and gold. EBR process components used to treat these waters included pretreatment for residual flotation reagents, a two-stage EBR with a 12-hour HRT, and anaerobic and aerobic posttreatment steps for residual BOD removal and re-aeration of treated waters.

Figure 37 shows the results of selenium removal during a 6-month period of bench-scale testing. Testing was divided into three periods. Period one ran from 1/30 to 3/3 for process validation. Period two ran from 3/4 to 4/18 and explored the effects of residual flotation reagents on selenium removal. Period three lasted from 4/19 to 6/18 and analyzed minimum nutrient requirements. Table 16 is a summary of the results in Figure 37. The anaerobic step further collected the selenium concentrated microbial cells from the EBR reactors and thus had a lower selenium effluent.

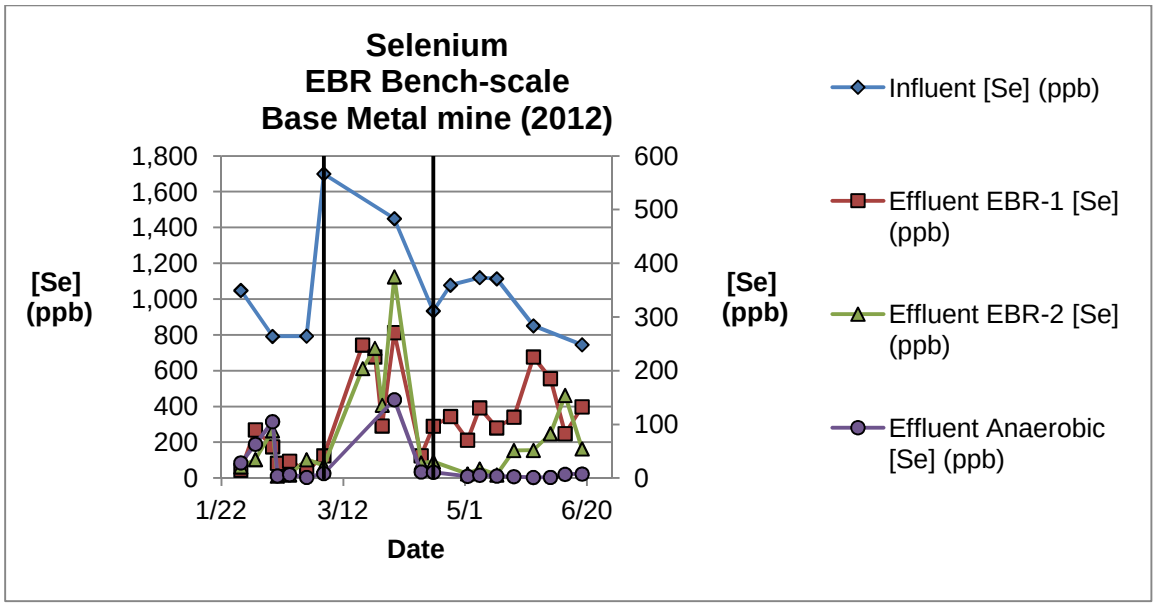

Figure 37. EBR bench-scale tests for the removal of selenium from the Base Metal mine water.

Table 16. Average selenium influent and effluent, EBR pilot Base Metal mine.

Date	Average influent selenium (ppb)	Average effluent selenium (ppb) EBR-1	Average effluent Selenium (ppb) EBR-2	Average effluent selenium (ppb) anaerobic treatment
1/30-6/18	1,057	121.7	36.9	6.7
1/30-3/3	878	39.42	31.4	10.1
3/4-4/18	1,361	146.01	148.43	44.24
4/19-6/18	982	127.74	52.23	4.12

Process effluent selenium concentrations fluctuated during bench-scale testing as different process components were evaluated for operational boundaries. Analysis to determine cause and effect is presented in the following graphs. Figure 38 shows the effect of residual flotation reagents on final effluent selenium. As can be seen, increases in flotation reagent concentration had a detrimental effect on selenium reduction and removal in the EBR system.

Figure 38 depicts the effect of breakthrough of flotation reagents in the pretreatment column on selenium removal. Residual flotation reagent concentrations were evaluated based on COD. Interference from residual flotation reagents on selenium removal was observed during

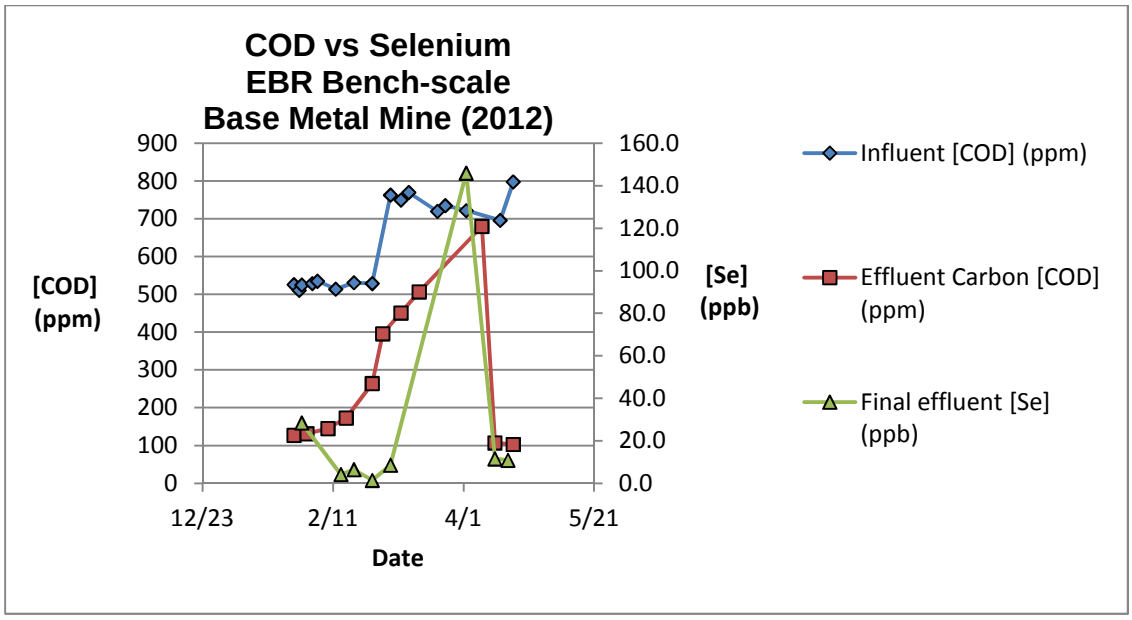

Figure 38. Effects of COD on selenium removal for bench-scale testing, Base Metal mine.

microbial screening tests. The results observed were used to establish organics loading capabilities of GAC for the removal of residual flotation reagents and the effect those reagents had on selenium removal performance. Breakthrough started to occur on 2/16 as carbon effluent COD increased from 127 ppm to 172 ppm. By 4/8, COD influent and carbon effluent were equal at 608 ppm. Between 3/4 and 4/2, final selenium effluent increased from 8.6 ppb to 146 ppb. The correlation coefficient between COD and selenium was calculated and the null hypothesis that there is no correlation between the two was tested. The z-statistic calculated value of 2.17 requires rejection and correlation is accepted.

Figure 39 examines the relationship between nutrient concentrations, influent selenium concentrations, and EBR system effluent selenium concentrations. Table 17 is a summary of the results in Figure 39. Figure 39 represents the nutrient concentration additions and selenium influent and effluent concentrations during bench-scale testing. Most notable in Figure 39 is the variability of influent selenium concentrations. The ability to successfully identify the proper nutrient ratio's given this high influent selenium variability proved difficult. Further complicating the analysis is the lag period between sampling and obtaining results. Nutrient addition optimization was evaluated at six different concentration ranges over the 6-month period.

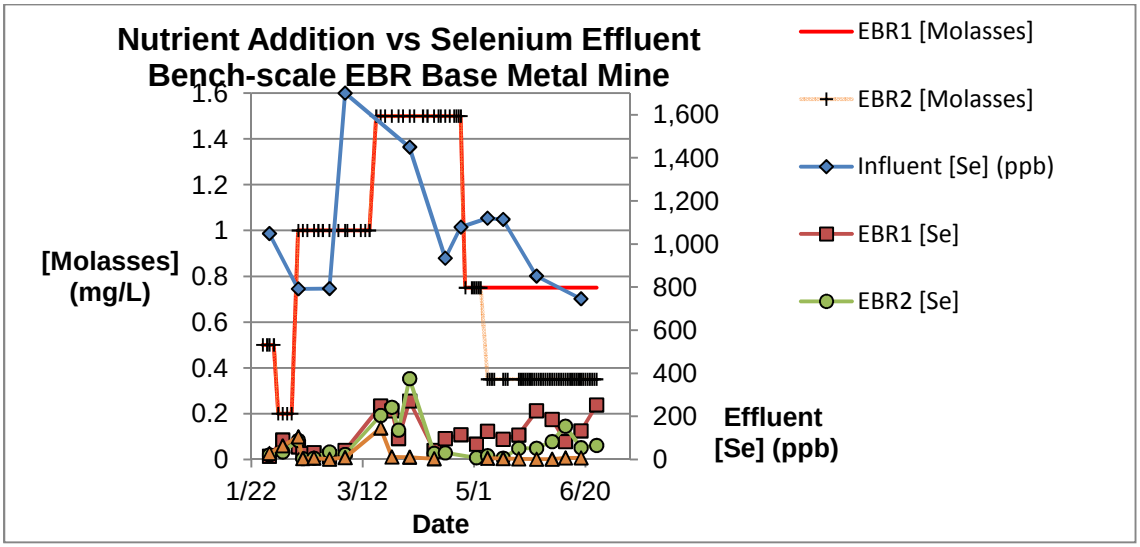

Figure 39. EBR bench-scale testing of nutrient concentration vs. selenium removal for Base Metal mine water.

Table 17. Nutrient concentration and final selenium effluent, EBR bench Base Metal mine.

Date	EBR-1 nutrient (ppm)	EBR-2 nutrient (ppm)	Final average EBR selenium effluent (ppb)
1/27-2/1	0.5	0.5	21
2/2-2/9	0.2	0.2	34.6
2/10-3/15	1	1	13.9
3/16-4/25	1.5	1.5	201
4/26-5/7	0.75	0.75	17.3
5/8-6/25	0.75	0.35	54.3

In analyzing the data, a clear trend emerges. Overall nutrient concentrations ranged from a low of 0.4 g/L to a high of 3 g/L. Ignoring the effects of residual flotation reagents breakthrough and changing influent selenium concentrations, results indicate optimum nutrient concentration is somewhere between 1.10 and 1.5 g/L. At an overall nutrient concentration of 2 and 1.5 g/L, EBR selenium effluent concentrations were below the target discharge of 20 ppb. Once overall nutrient concentrations fell below 1.5 g/L in the EBRs, selenium effluent rose above the target discharge.

However, the anaerobic process step was able to handle increased selenium loading and final effluent remained below the target discharge until the end of testing on 6/18. The final nutrient concentration tested for EBR1 was 0.75 g/L and EBR2 was 0.35 g/L.

Figure 40 shows the ORP data collected during bench-scale tests. The target ORP for the EBR reactors was between -150 mv to -250 mv, but fell below the targeted range toward the end of the test and was a reflection of the nutrient adjustment rather than the direct electron addition. Nutrient addition was continually varied in each reactor; however, due to the applied voltage, ORP values remained more stable throughout the majority of the test and standard deviation remained below 50 mv.

Base Metal Mine Results; Pilot-scale

Pilot-scale testing at the Base Metal mine was completed over two summers with treatment system adjustments made to meet changing mine wastewater conditions. EBR process components used were developed from bench-scale testing. Influent water chemistry exhibited

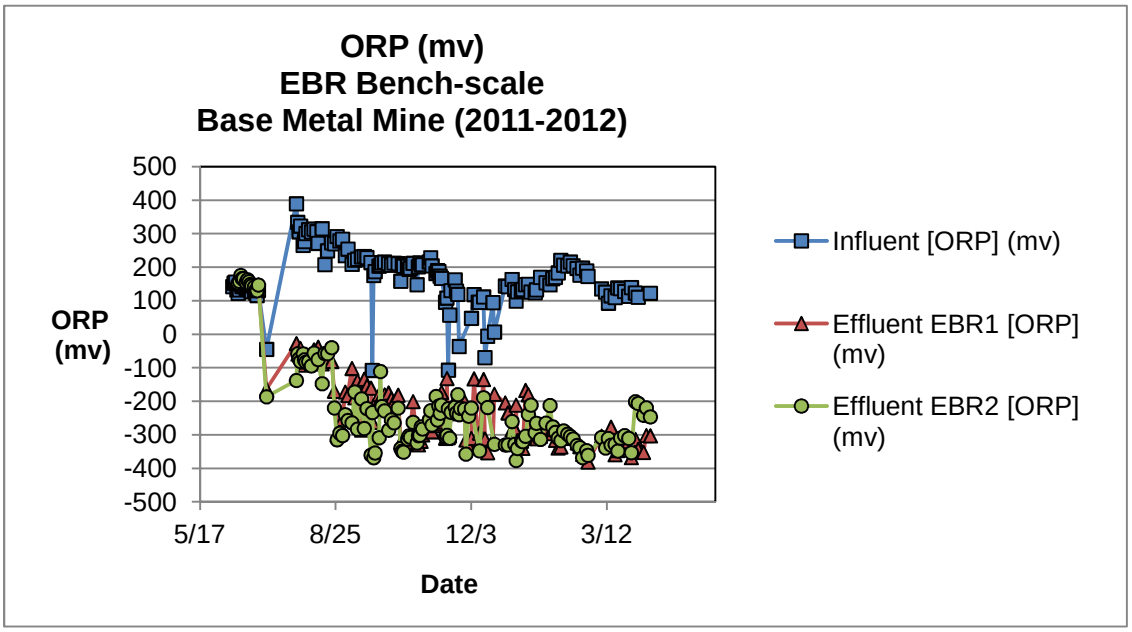

Figure 40. ORP data for EBR bench-scale, Base Metal mine.

dramatic fluctuations in selenium concentrations and residual flotation reagents requiring large engineering margins for each process component. Results presented below represent the data collected during two summers of pilot tests using different EBR configurations. Figure 41 presents the selenium removal results from EBR pilot-scale testing from the Base Metal mine in 2011.

During pilot-scale testing, several issues arose that required EBR operation to be adjusted. A major issue effecting the 2011 pilot-scale testing was influent water quality fluctuations. Influent water was collected from three different places during the 2011 pilot-scale test and the curve depicting selenium influent concentrations reflects those changing conditions. On 7/13, EBR pilot system influent water was collected from the make-up tank for the flotation circuits located in the ore processing mill. Due to a miscommunication, two EBR influent water samples were collected from a nearby creek on 7/20 and 7/25.

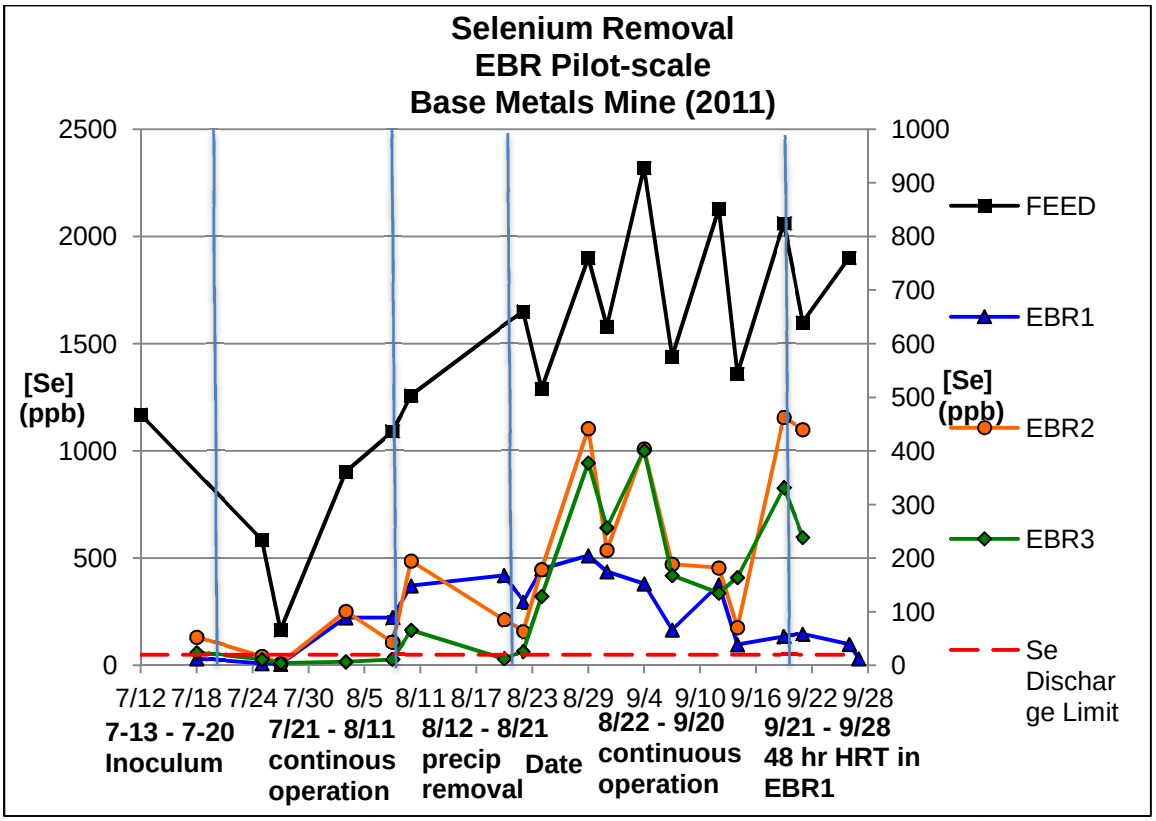

Figure 41. EBR pilot-testing for Base Metal mine waters.

On 8/7, a sample of water was collected from the mill shotcrete collection basin pump-back that caused a calcium precipitate to form throughout the EBR system. In an effort to evaluate process upsets and EBR system recovery, 300 gallons of clean water adjusted to pH 4.0 was prepared and used to flush and clean the precipitates in EBR-1. Following flushing of EBR-1, nutrient was added to an equal volume of pH 7 water, pumped into EBR-1, and allowed to sit for 3 days in an effort to reestablish the microbial population. This proved effective and EBR-1 recovered with time and was producing discharge quality water with a retention time representative of the three-stage EBR system. EBRs 2 and 3 were not cleaned with pH 4 water to study their ability for self-recovery; they did not recover and selenium removed by these reactors before the upset was gradually released.

Because the make-up water for the flotation circuits is constantly being changed for Base Metal recovery from different ore bodies, it was decided to collect the EBR influent water from the pump-back house located next to the mine/s tailings dam. The tailings dam is approximately 6 million gallons sediment and water retention pond used for mine site sediments and water collection/storage. Operation of the system continued from 8/20 to 9/20 using water from the pump-back house; influent water continued to show high variations in selenium concentrations due to short-circuiting in the tailings dam. On 9/22, EBR-2 and -3 were taken off-line and influent flow rate through EBR1 was adjusted to 36 hours; the system was operated in this configuration until freeze-up on 9/28.

Quality control on sample analysis was conducted during the 2011 EBR pilot operation using duplicate samples sent to two different laboratories and sample dilutions. There was a significant difference in analysis results between the two labs. The highest difference was 159%, the lowest was 3.7%, and the average difference was 53%. EPA guidelines require ICP-MS for metals analysis, but the confidence in data quality, as indicated in Table 18, is elusive at best. Figure 42 is the data collected from EBR pilot-scale testing at the Base Metal mine in 2012.

Based on the 2011 variation in influent water selenium concentrations, an additional 5,000 gallon EQ tank was set up to be filled and drained at the same rate in 2012; this additional EQ tank dampened influent selenium concentration fluctuations. With a more consistent influent

Table 18. Quality control test for selenium, EBR pilot; Base Metal mine, 2011.

Date	Sample	Certified Lab 1 [Se] (ppb)	Certified Lab 2 [Se] (ppb)	Average % Difference
8/4/2011	Feed	903	3030	54%
	EBR1	222	297	29%
	EBR2	101	195	64%
	EBR3	7	34	132%
8/23/2011	Feed	1650	1250	27.5%
	EBR1	296	256	14.5%
	EBR2	63	19	54%
	EBR3	26	3	159%
9/5/2011	Feed	1580	1870	17%
	Feed- duplicate	1830	1900	3.7%
	EBR1	437	296	38.5%
	EBR1 - duplicate	225	303	29.5%
	EBR1 - triplicate	223	309	32%
	EBR1 - 1:2 dilution	101	171	51.5%
	EBR1 - duplicate of 1:2 dilution	100	172	53%
	EBR3	257	100	88%
	EB3 - duplicate	190	98	64%
	EBR - 1:2 dilution	31	46	39%

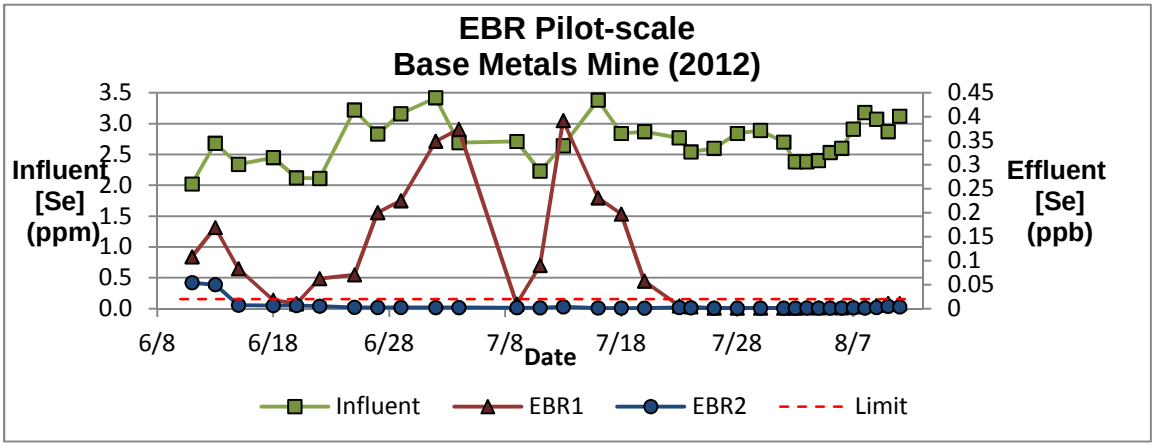

Figure 42. EBR pilot-scale testing for Base Metal mine water.

selenium concentration, the two-stage EBR process delivered more consistent effluents. Analysis of EBR-1 results shows dramatic changes in effluent selenium concentrations. As previously noted, residual flotation reagents present in the influent interfered with selenium removal and those effects are strongly manifest in EBR-1. In an effort to evaluate different reagent removal solutions, both GAC and GAC with microbes were tested as pretreatment steps to remove and degrade the organics. As can be seen in Figure 42, EBR-1 buffered the effect of the flotation reagents and EBR-2 was unaffected and removed selenium to an average of 0.002 ppm throughout the entire 2012 test. 2012 quality control samples results are shown in Table 18.

The QC included three high range samples (i.e., where the expected selenium concentration exceeded 500 ppb) and six low range samples (i.e., where the expected selenium concentration did not exceed the discharge limit of 20 ppb). Samples were diluted two- and four-fold with deionized water. Results presented in Table 19 are satisfactory in the low selenium range, which was the focus of this research. Controls of low range selenium samples showed good match with the expected values, with standard deviation ranging between 0.14 and 0.40 ppb, corresponding to a relative deviation of 0.87-9.44%. High range selenium samples returned significantly poorer QC results, with relative deviation of 5.4-36.4%. It is worth noting that all the selenium quality control concentrations were higher than the expected values, based on the undiluted sample analysis results. The first pretreatment solution examined, on mine management request, was a GAC sorption column; results are shown in Figure 43 from testing conducted from 6/11 to 7/2. From 6/11 to 6/20 reagent removal by GAC performed well and EBR-1 performance continually improved. However by 6/22, EBR-1 started to experience decreasing performance and by 7/4, selenium removal was reduced from 95% to 89%. An analysis of COD over the same period depicts a COD breakthrough on 6/25 that corresponds to the decrease in selenium removal in Figure 42.

On 7/2, 300 gallons of EBR-1effluent water and nutrient was added to the GAC tank to develop an inoculum for a new microbial pretreatment step to degrade the flotation organics. The inoculum was allowed to establish a biofilm on the GAC for 3 days. The EBR system was again

Table 19. Quality control results for selenium, EBR pilot; Base Metal mine, 2012.

Sample and dilution factor	Results (ppb)	Expected Results (ppb)	Standard deviation	% of standard deviation
EQ duplicate	2,520	2,340	127.28	5.44%
EQ (1:2 dilution)	1,470.00	1,170.00	212.13	18.1%
EQ (1:4 dilution)	886.00	585.00	212.84	36.4%
EBR-2 duplicate	7.75	7.19	0.40	5.56%
EBR-2 (1:2 dilution)	3.79	3.60	0.14	3.89%
EBR-2 (1:4 dilution)	2.04	1.80	0.17	9.44%
AE duplicate	16.20	16.00	0.14	0.87%
AE (1:2 dilution)	8.57	8.00	0.04	5.00%
AE (1:4 dilution	4.42	4.00	0.30	7.50%

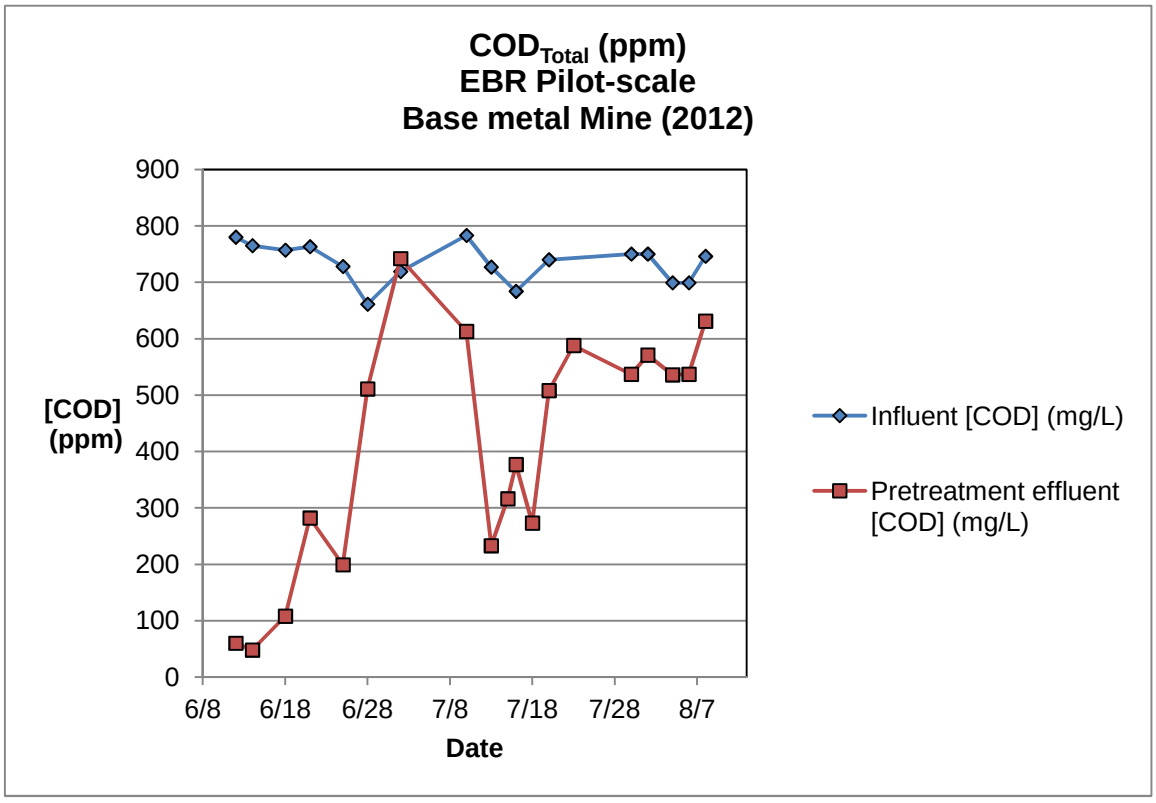

Figure 43. COD influent and pretreatment effluent.

started on 7/6 and selenium removal from EBR-1 improved initially and then decayed. However by 7/16, selenium removal performance did return as the pretreatment microbial population was established. EBR-1 effluent selenium concentrations returned to about 2 ppb and continued through the end of testing on 8/11. The COD effluent values remained high throughout the remainder of the test period. The reason selenium performance improved after inoculation is due to the transformation of residual organic flotation reagents into more suitable carbon forms for utilization by the selenium reducing microbial population. This allowed the normally added nutrients to be substantially reduced.

Figure 44 depicts ORP measurements during pilot-scale testing in 2011. Important in this graph is the influent ORP. Fluctuations from 200 mv to -300 mv were observed in the influent. Ore milling operations require pH and chemistry adjustments to create proper conditions for ore recovery. Fluctuating ORP values in influent waters are artifacts from chemical additions by mill personnel and intermittent short-circuiting in the tailings dam. High ORP variability in the influent poses a problem of ORP control within the bioreactor.

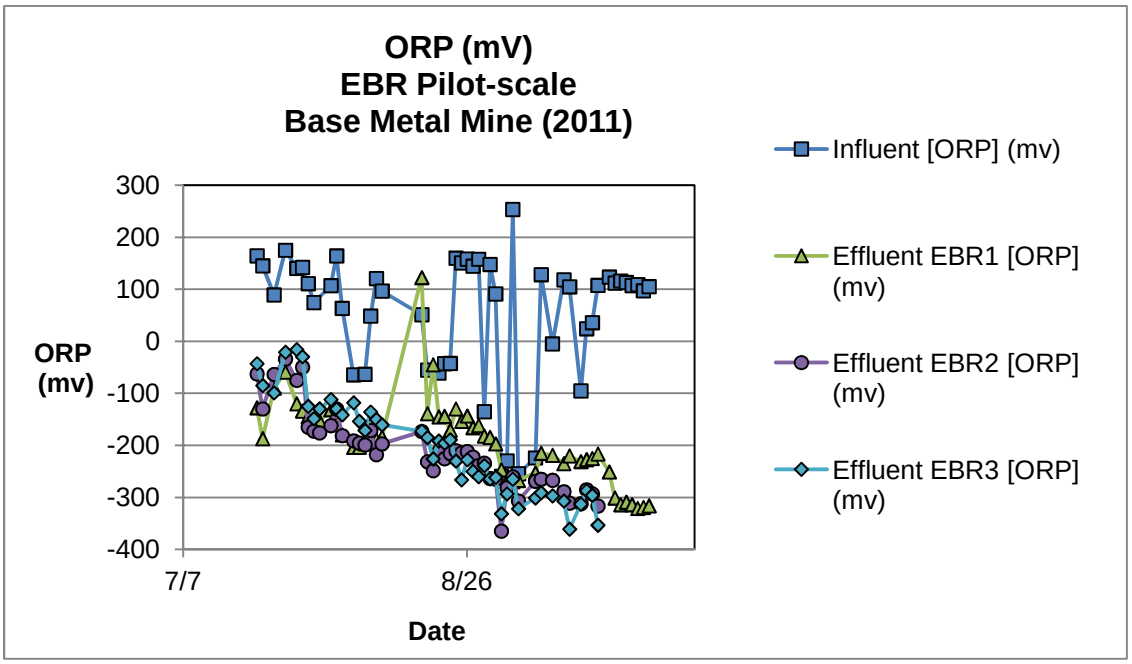

Figure 44. ORP data for EBR pilot-scale Base Metal mine water.

The addition of directly supplied electrons provides reactor ORP stability as seen in Figure 44. This stability is still trending downward due to excess organics now being supplied through flotation reagent degradation and slow adjustments made to the normally added molasses nutrient.

Figure 45 is ORP data measurements during the 2012 pilot-scale testing; high variability in influent ORP was observed. Influent ORP values fluctuated from a high of 60 mv to a low of -364 mv over the test period in 2012. Typical responses by bioreactor operators to this are the addition of chemicals to stabilize the reactor ORP. The EBR overcomes this ORP variability with directly supplied electrons as opposed to chemicals. Direct addition of electrons is more efficient because it does not require chemical metabolism or conversion to make the electrons available within the system. Chemical additions for ORP are difficult to control due to inefficient mixing, kinetics, and variable influent ORP.

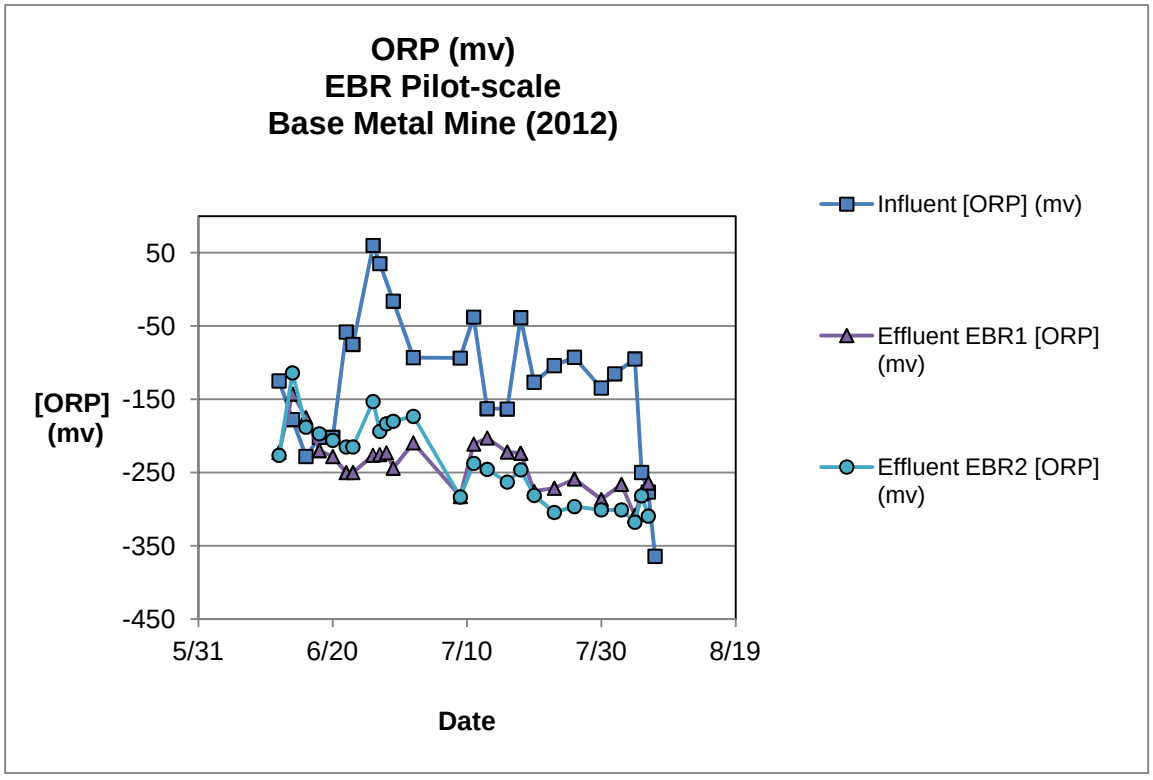

Figure 45. ORP data for EBR pilot-scale Base Metal mine water.

During pilot-scale testing in 2011, the effects of temperature on selenium removal performance were evaluated twice and are presented in Figure 46. On 7/23, the water heater for the EBR influent was turned off. EBR influent water temperature dropped from 15.9^0 C to 7.1^0 C over 9 days. Effluent selenium concentrations increased from 11 ppb on 8/4 to 66 ppb on 8/9. However, final selenium effluent improved to 121.1 ppb by 8/11 as the microbes acclimated to the lower temperatures. Again on 9/20, the water heater was turned off and influent water temperature dropped from 7.6 C to 2.1 C. At the same time, EBR-2 and EBR-3 were taken off line and the HRT in EBR-1 was increased to 48 hours.

In Figure 47, the data collected to examine the effect of temperature on EBR selenium removal are presented. Results indicate that temperature was not a significant factor for EBR selenium removal at temperatures 7.7^0 C and above.

Additionally, the EBR system's ability to remove other metals and inorganics is shown in Table 20. The following table presents results obtained from the Base Metals mine water treatment pilot tests.

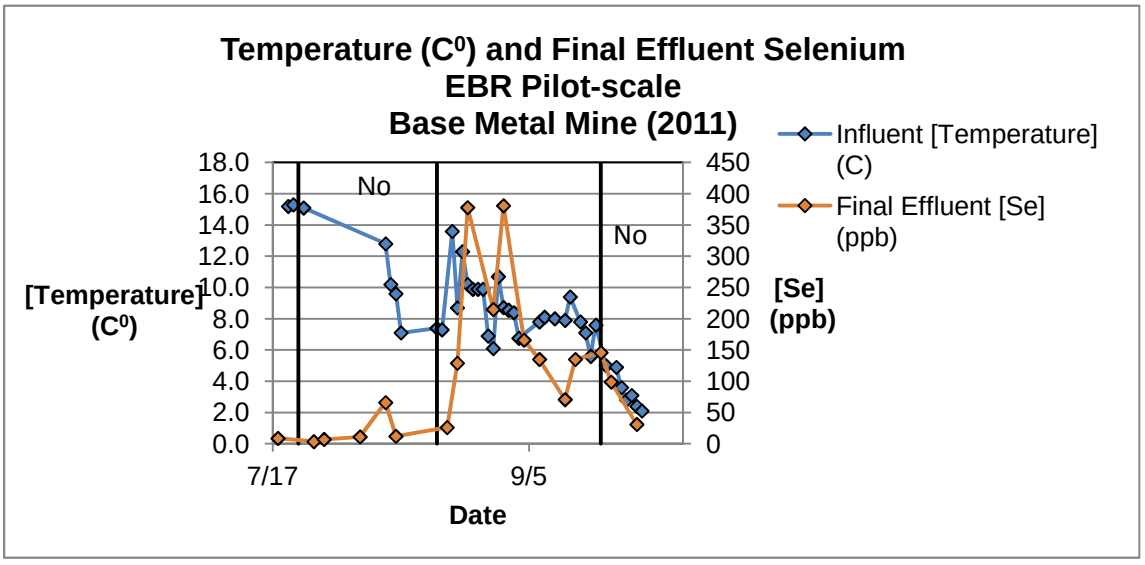

Figure 46. Temperature and final selenium effluent concentration, EBR pilot-scale for Base Metal mine water.

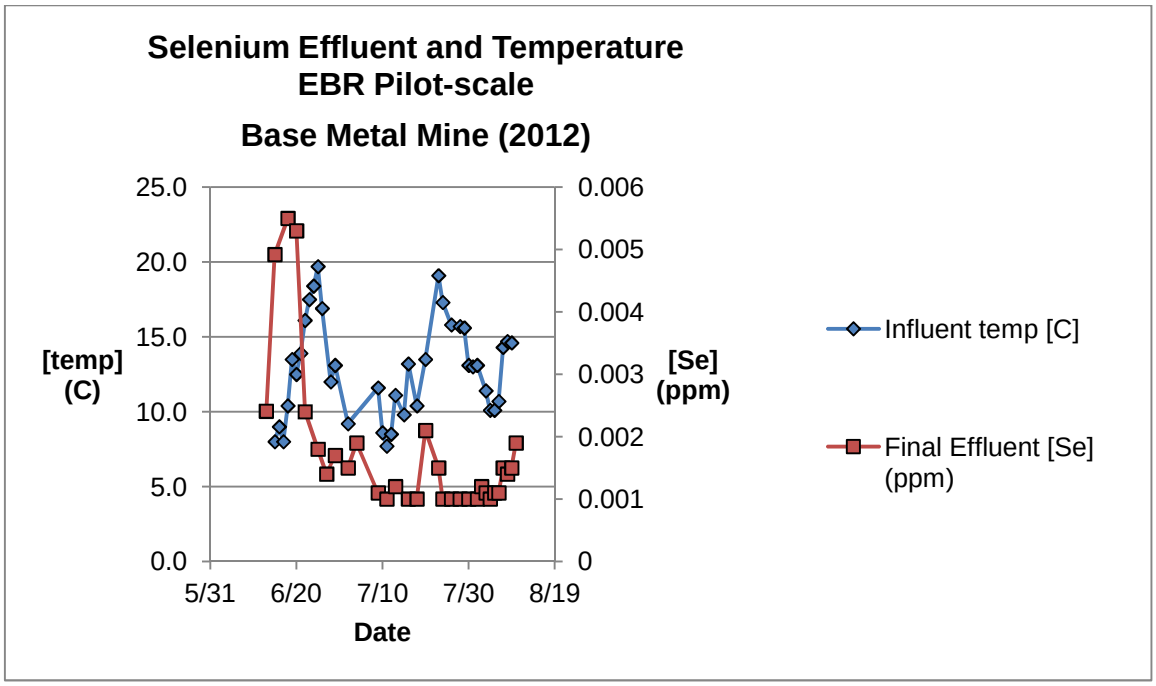

Figure 47. The effects of temperature on selenium removal.

Table 20. Suite of metals analyzed and influent and effluent concentrations.

Parameter	Average Influent (ppm)	Average Discharge (ppm)	% Removal
Antimony	0.15	<0.001	>99.3%
Cadmium	0.014	<0.0002	>98.0%
Copper	0.41	<0.005	>98.7%
Lead	0.30	<0.0008	>99.7%
Molybdenum	0.10	<0.0005	>99.5%
Selenium	2.73	<0.002	>99.9%
Silver	0.041	<0.0001	>99.8%
Zinc	0.46	<0.03	>93.5%
Nitrate-N	3.3	<0.1	>97.1%
Nitrite-N	0.9	<0.02	>97.8%
Cyanide $_{WAD}$	0.26	<0.005	>98.1%
Cyanide $_{TOTAL}$	0.47	<0.005	>98.9%

CONCLUSIONS

Specific to Research

The results presented in this thesis support previous research showing that directly applied electrons can improve microbial and microbial biofilm robustness (growth and survival) and microbial transformation kinetics. This research provides an accounting of how the objectives have been met and presents original contributions to the research and development of the EBR technology.

This research has demonstrated that directly applied electrons, in a bioreactor environment using actual mining influenced wastewaters, are capable of improving bioreactor performance for the removal of nitrate, arsenic, and selenium. Additional research contributions are presented below.

Screening and selecting the appropriate microbes facilitated removal of nitrate and arsenic from Gold mine waste and selenium removal from Base Metal mine water influencing both contaminant concentrations and removal kinetics. The ability to identify the proper microorganisms, develop and maintain appropriate population densities, and develop appropriate biofilms, for the transformation of target contaminants improved EBR and bioreactor performance in general.

Screening and selecting different MSM for removal of nitrate and arsenic in a closed heap-leach Gold mine and selenium in Base Metal mines waters influenced contaminant concentrations and removal performance. MSM screening and selection of the appropriate microbial support materials are also important in bioreactor function and performance. Analysis of MSM materials, for potential leaching of contaminates in a bioreactor, is required before selection in order to achieve the best biofilm stability and contaminant removal kinetics.

Bench-scale testing of the electro-biochemical reactor or EBR versus a conventional bioreactor or CBR demonstrated a 13% improvement in arsenic removal. The EBR removed arsenic from an average of 350 ppb to an average of 12 ppb while the CBR was only able to achieve an average of 50 ppb effluent. The statistical relevance of these results was better than 99%. Nitrate-N was completely removed to nondetectable levels.

EBR performance was evaluated on-site at the Gold mine in a pilot-scale system at flow rates of up to 4 L/minute, a 24-hour HRT, arsenic concentrations of up to 800 ppb, and temperature fluctuations of up to 8.8^0 Celsius with no degradation in performance. Final effluent arsenic concentrations averaged 50 ppb. Nitrate-N was completely removed in less than 12 hours.

Further work included bench- and pilot-scale EBR testing of waters from a Base Metal mine containing selenium influent concentrations ranging from 1.8 to 5 ppm. In bench-scale tests, the EBR removed selenium from an average of 3.5 ppm to an average of less than 5 ppb within 12 hours. In pilot-scale tests, selenium was removed from an average influent concentration of 2.73 ppm to an average effluent of 0.002 ppm. Flexibility in process design at both bench- and pilot-scale was required to address changing influent water chemistry and contaminant concentrations.

Bench- and pilot-scale testing of the heap-leach Gold mine and the Base Metal mine waters provided information on EBR system process step, nutrient, voltage, and HRT requirements necessary to meet discharge targets. Bench-scale validation for target contaminate removal of a particular site's waters is necessary to define engineering requirements for pilot-scale testing. Water chemistry components and fluctuations influence process steps required and overall treatment system design. Proper process selection for a given water chemistry component requires evaluation of different methods. System capability and flexibility at both bench and lab-scale provides data for analysis and optimal wastewater system design.

Benefits to Industry

A more cost effective solution for the removal of nitrate, arsenic, and selenium can be achieved using the Electrochemical Bioreactor. The ability of the EBR system to meet discharge quality of MIW waters using endemic microbes at temperatures below 4º C provides the mining industry with a better solution to meet ever expanding COCs, ever decreasing acceptable effluent concentration levels, and ever increasing effluent waters. The direct addition of electrons reduces nutrient requirements, improves ORP control and stability, and increases bioreactor robustness required to meet discharge requirements.

Additionally, the EBR systems ability to remove other metals and inorganics is shown in Table 21. The following table presents results obtained from the Base Metals mine water treatment pilot tests.

Table 21. Suite of metals analyzed and influent and effluent concentrations.

Parameter	**Average Influent (ppm)**	**Average Discharge (ppm)**	**% Removal**
Antimony	0.15	<0.001	>99.3%
Cadmium	0.014	<0.0002	>98.0%
Copper	0.41	<0.005	>98.7%
Lead	0.30	<0.0008	>99.7%
Molybdenum	0.10	<0.0005	>99.5%
Selenium	2.73	<0.002	>99.9%
Silver	0.041	<0.0001	>99.8%
Zinc	0.46	<0.03	>93.5%
Nitrate-N	3.3	<0.1	>97.1%
Nitrite-N	0.9	<0.02	>97.8%
Cyanide $_{WAD}$	0.26	<0.005	>98.1%
Cyanide $_{TOTAL}$	0.47	<0.005	>98.9%

Recommendations for Future Research

Upon completion of the objectives of this thesis and based on lessons learned from the pursuit of those objectives, it is suggested that several related areas are worth further research. The following is a list of those suggestions.

- In development of the pilot-scale EBR system, several different electrode materials were tested, including iron, stainless steel, and titanium. Although minimal corrosion was experienced with stainless steel and titanium, other electrode materials such as platinum and protective coatings for the electrodes were not tested. For titanium electrodes, an iridium oxide-tantalum oxide coating is suggested. If a platinum electrode is tested, a rhodium-palladium coating is suggested.
- Optimization of ORP for specific donor/acceptor reactions plays a key role in EBR performance. A potential strategy to control ORP that can be tested is the on/off switching of the applied voltage. Experiments to quantify the effects of an intermittent voltage could be pursued.
- Further research with respect to ORP control should include the evaluation of variable voltage input. Voltage is the electrical potential energy of an electron per unit charge and quantum theory states that electrons associated with a certain molecule can only absorb discrete quanta. Based on these two concepts, it only follows that tuning the voltage of the EBR system to the specific energy of a specific molecule will allow for absorption of that electron into the molecule. As described in Chapter 2 under the section Electro-Biochemical Reactor; three different potential mechanisms of electron transfer were suggested: direct electron transfer through c-Type Cytochromes, through intermediaries such as humic acid, and immobilized structures such as nanowires. By tuning the voltage to the correct quanta associated with the cytochrome, intermediary, or nanowire, electron transfer can be optimized.
- Further suggestions for potential research could include the exploration of a control system to target specific ORPs. Influent ORP varies both from site to site and during site testing; optimal ORP for specific contaminant transformations is necessary; by

measuring the influent ORP and the ORP within the reactor, a potential feedback loop can developed to adjust voltage potential to account for changes in influent ORP, reactor ORP, and target ORP.

APPENDIX A

GRAPHS OF ALL DATA COLLECTED FROM EBR BENCH-SCALE TESTING OF GOLD MINE WATERS

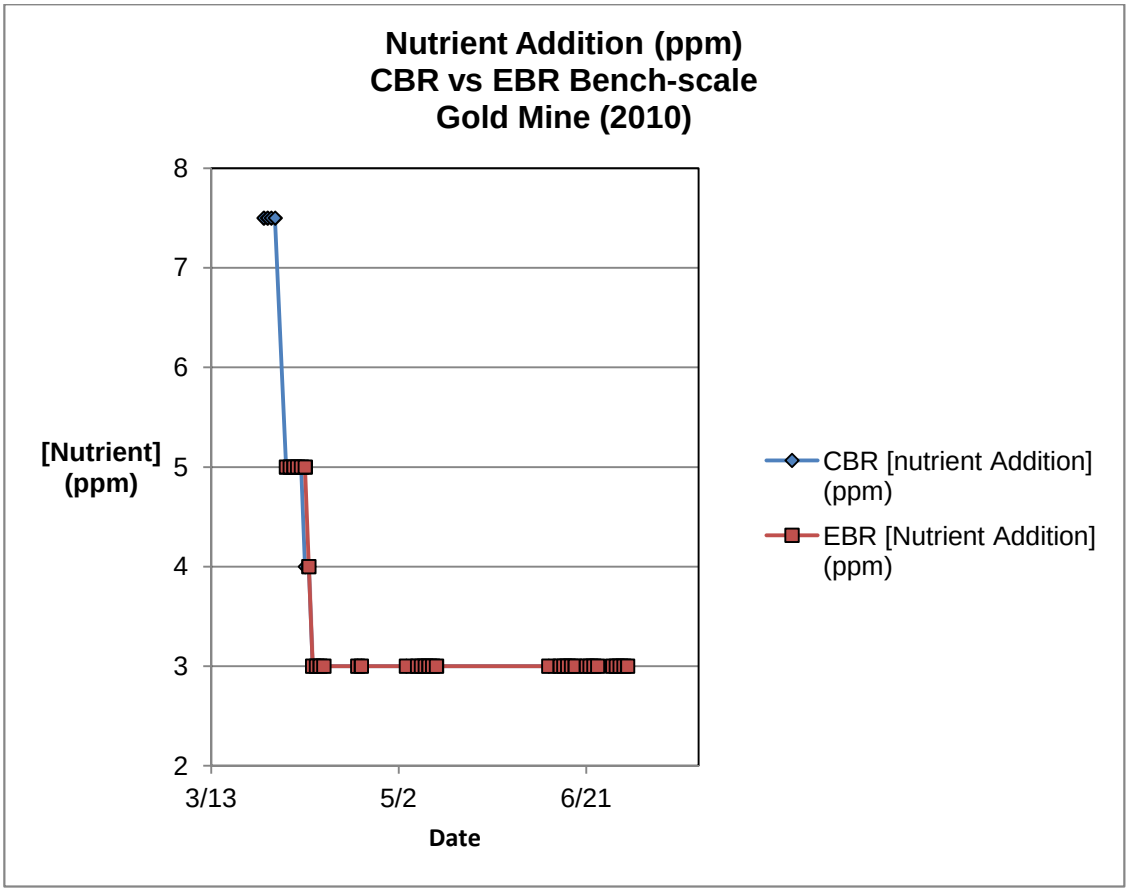

Figure A1. Nutrient Addition CBR vs. EBR Bench-Scale.

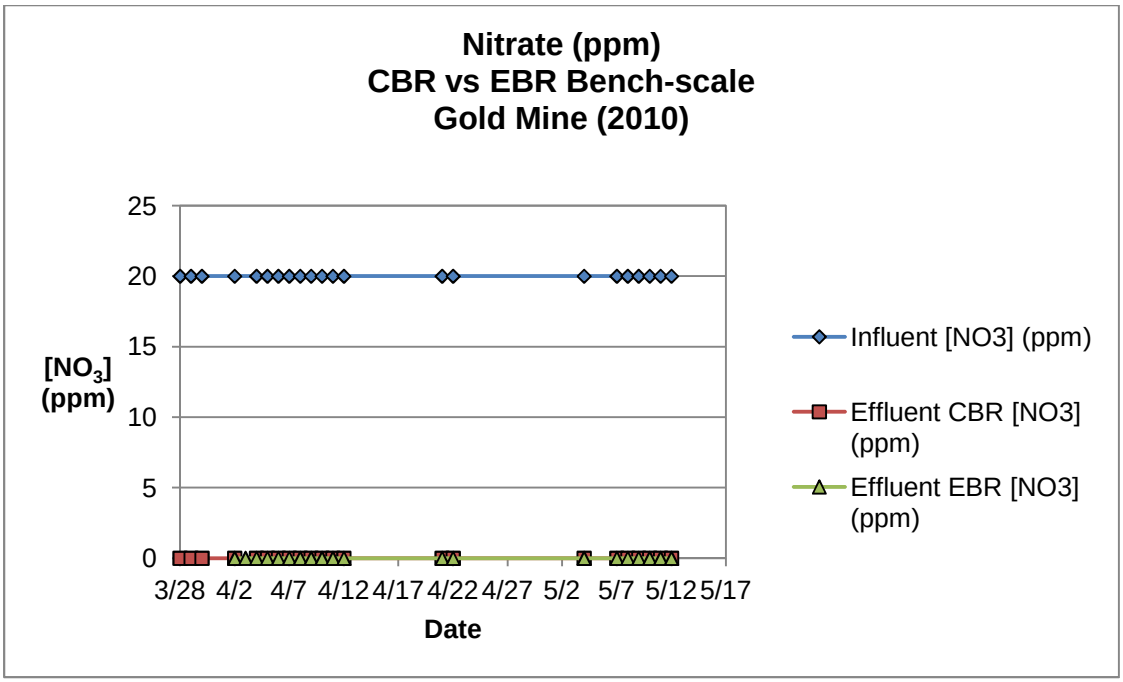

Figure A2. Nitrate CBR vs. EBR Bench-Scale.

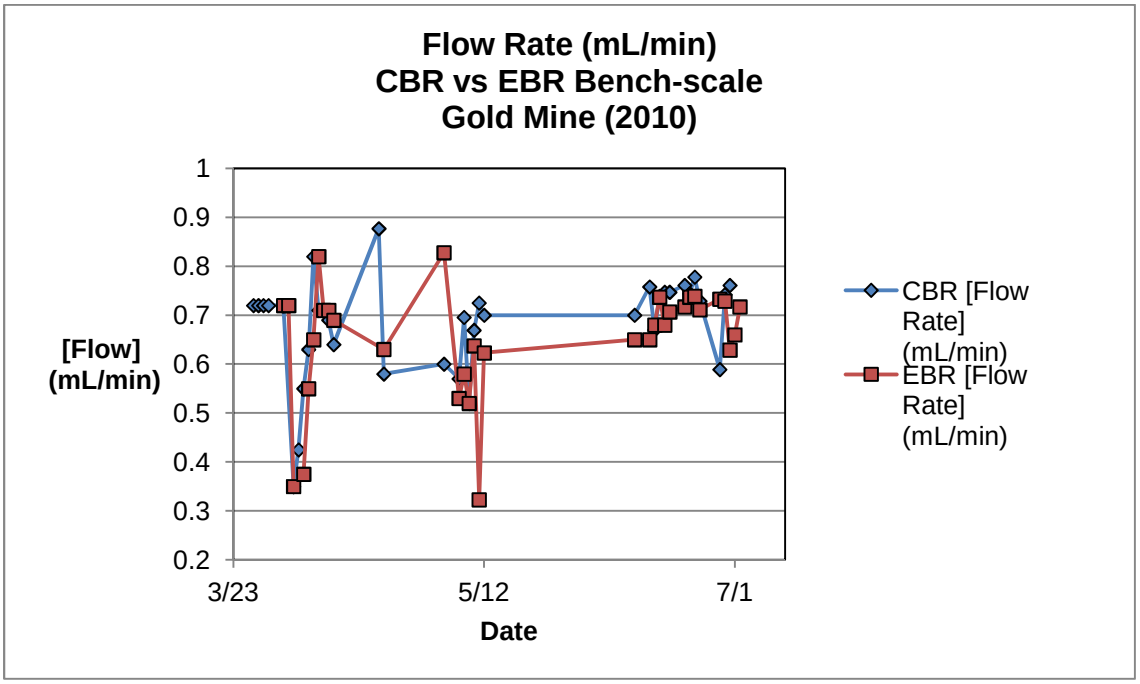

Figure A3. Flow Rate CBR vs. EBR Bench-Scale.

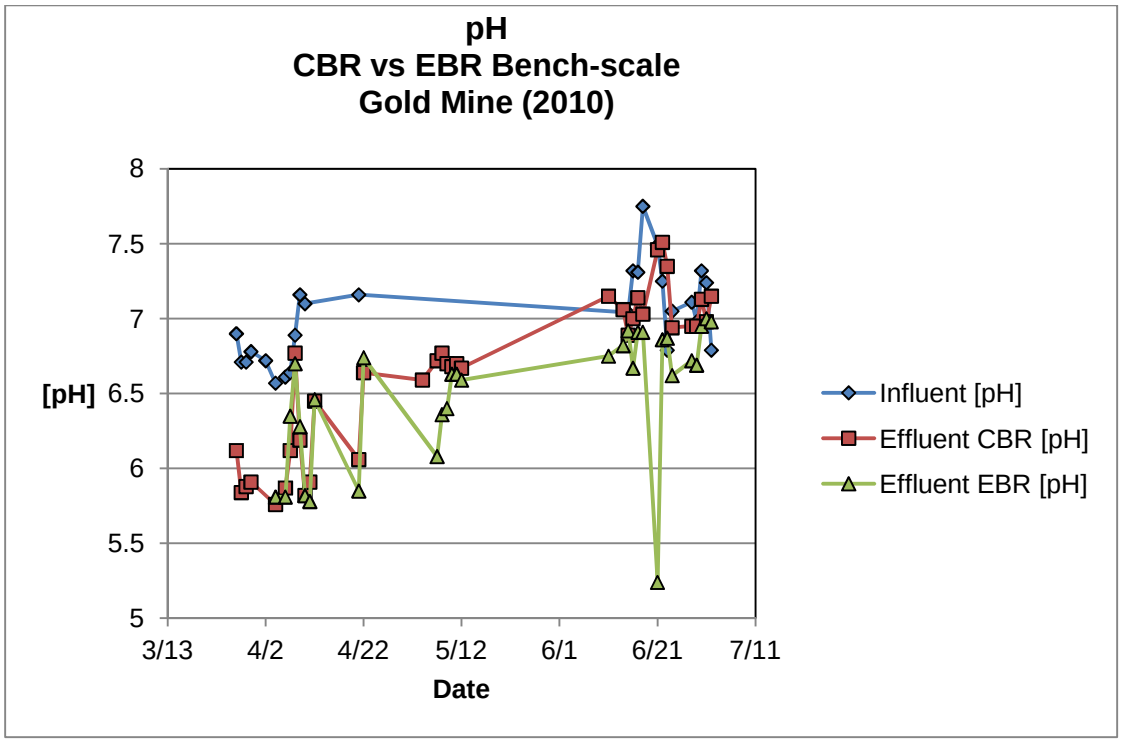

Figure A4. pH CBR vs. EBR Bench-Scale.

APPENDIX B

DATA AND GRAPHS FROM EBR PILOT-SCALE TESTING OF GOLD MINE WATER

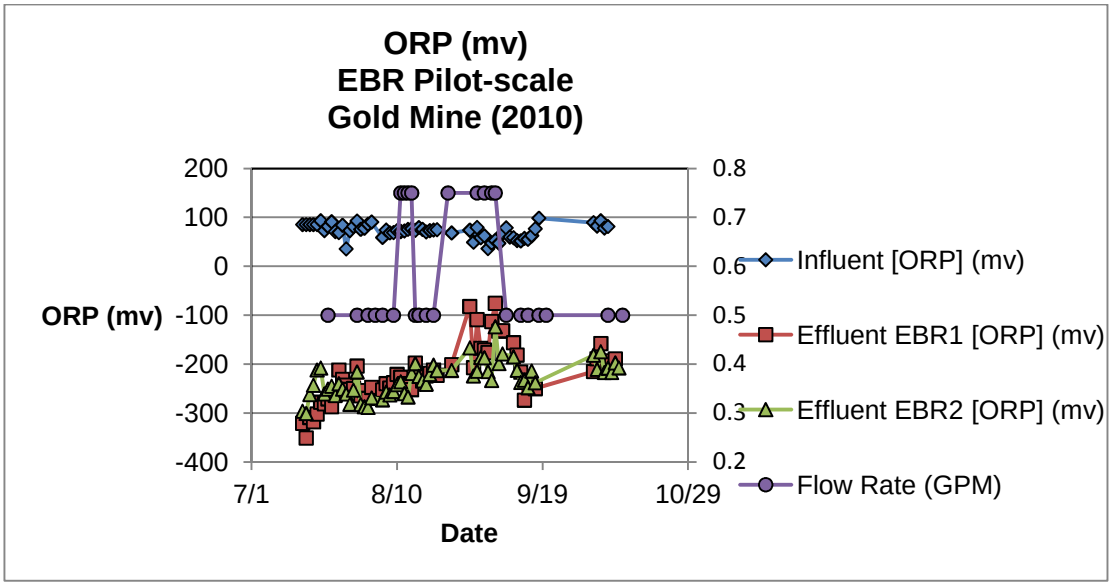

Figure B1. ORP EBR Pilot-Scale (2010).

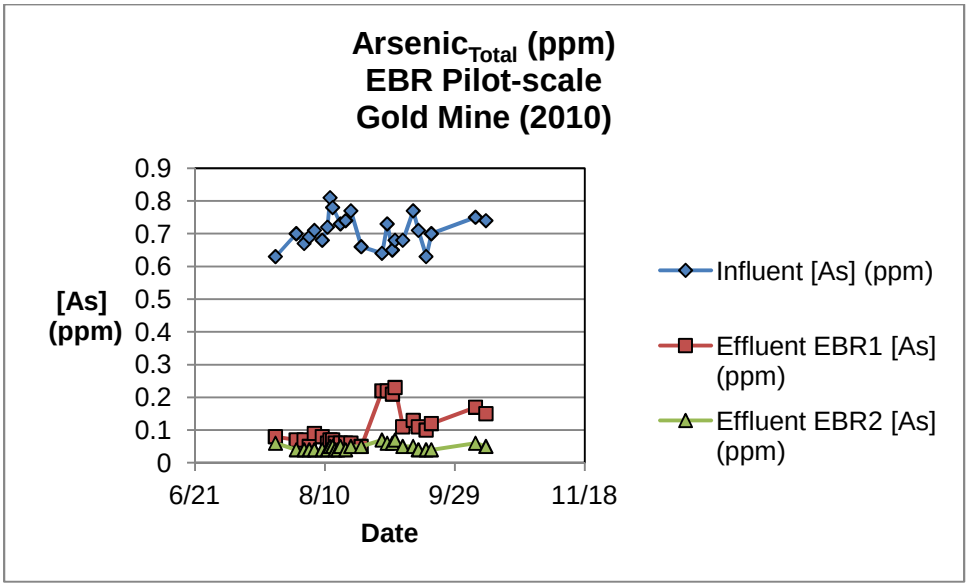

Figure B2. Arsenic$_{Total}$ EBR Pilot-Scale (2010).

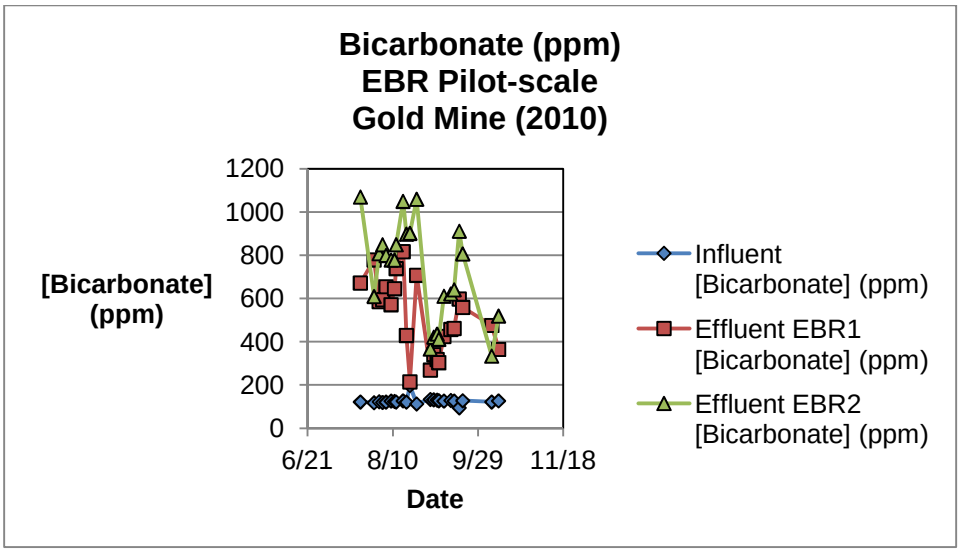

Figure B3. Bicarbonate EBR Pilot-Scale (2010).

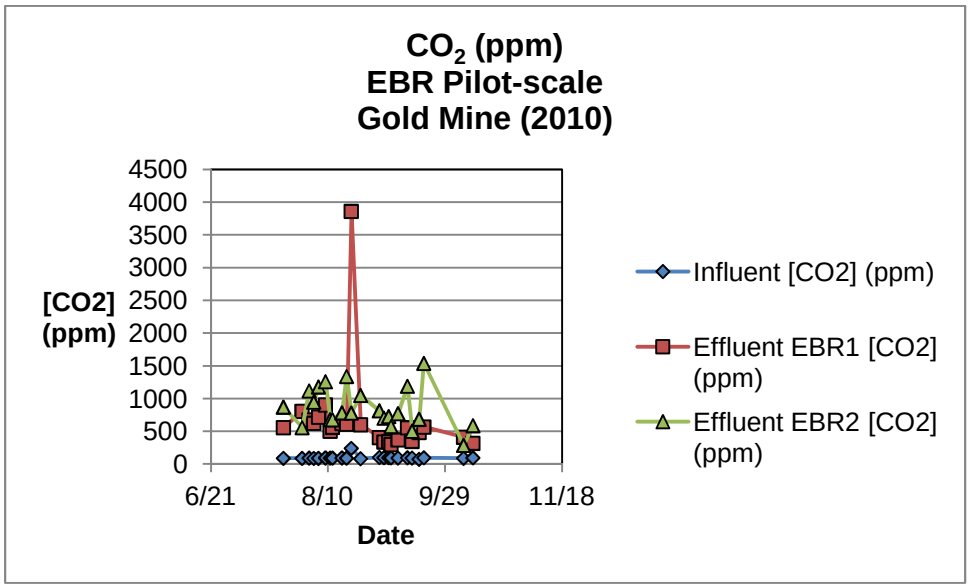

Figure B4. CO_2 EBR Pilot-Scale (2010).

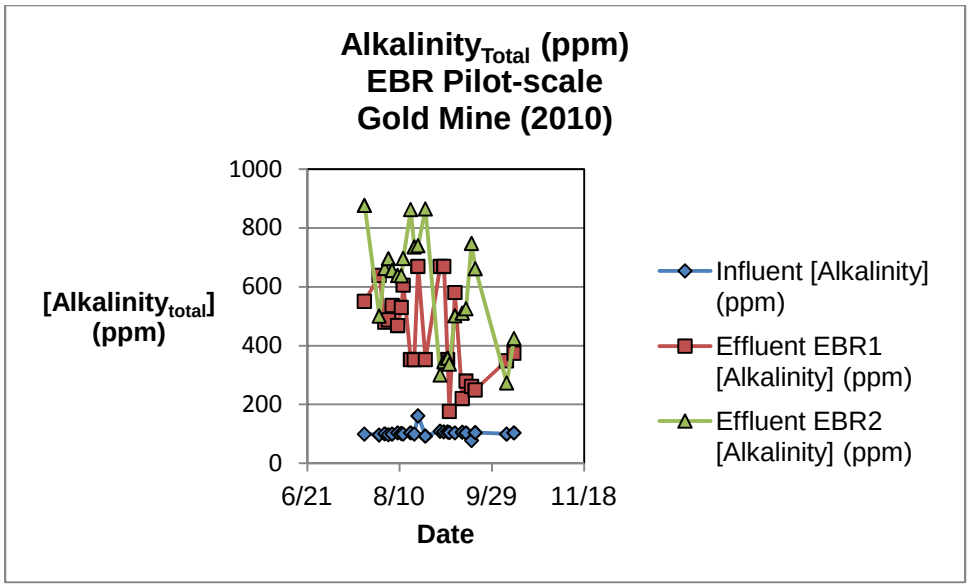

Figure B5. Alkalinity$_{Total}$ EBR Pilot-Scale (2010).

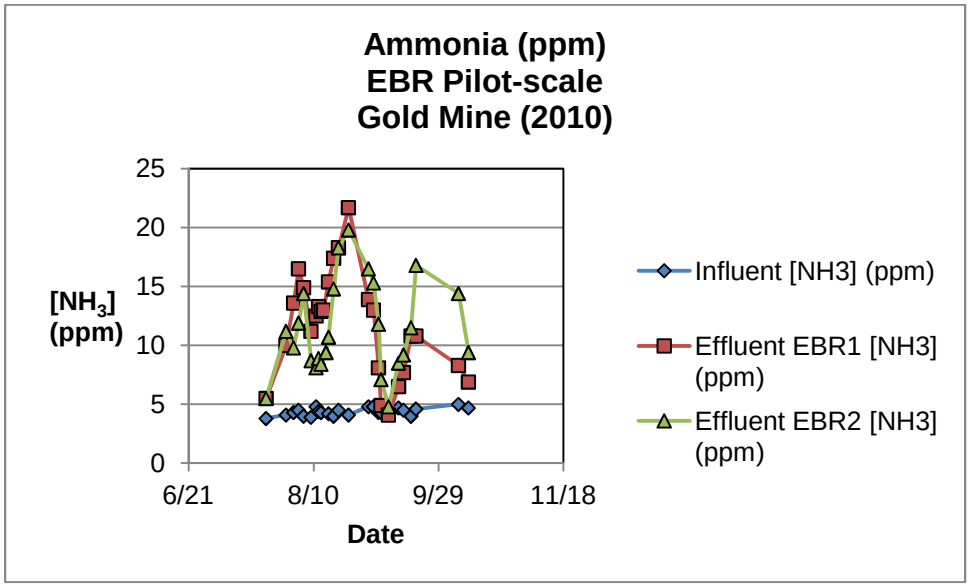

Figure B6. Ammonia EBR Pilot-Scale (2010).

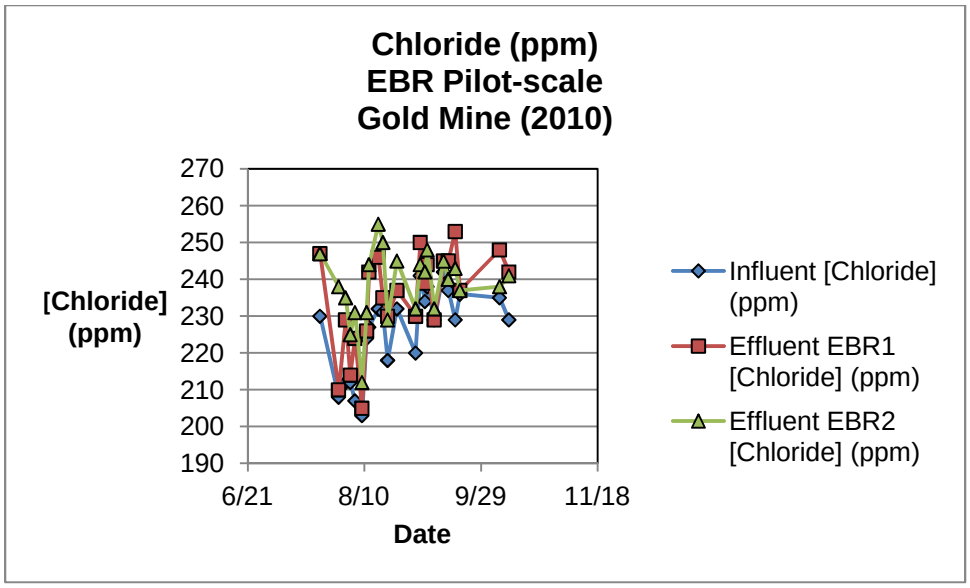

Figure B7. Chloride EBR Pilot-Scale (2010).

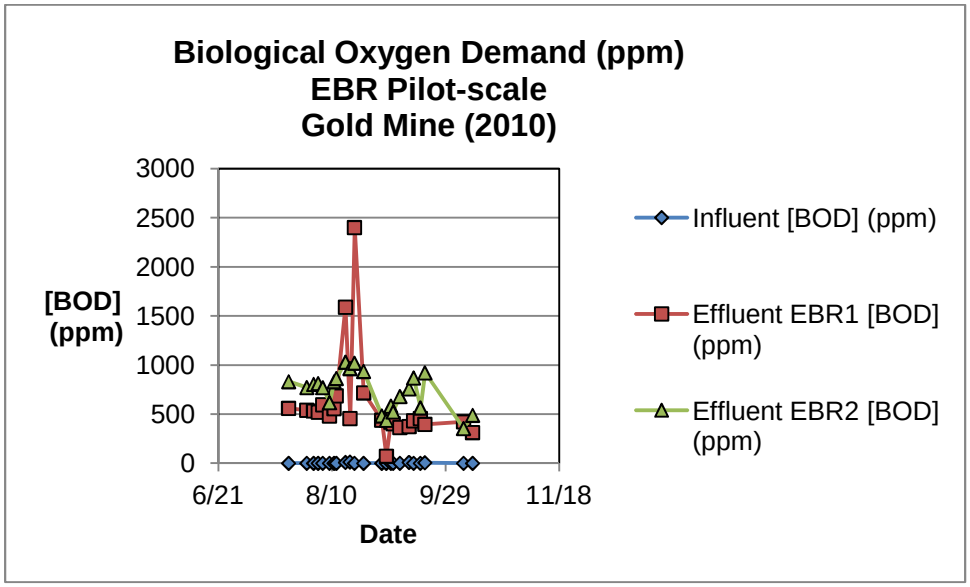

Figure B8. Biological Oxygen Demand EBR Pilot-Scale (2010).

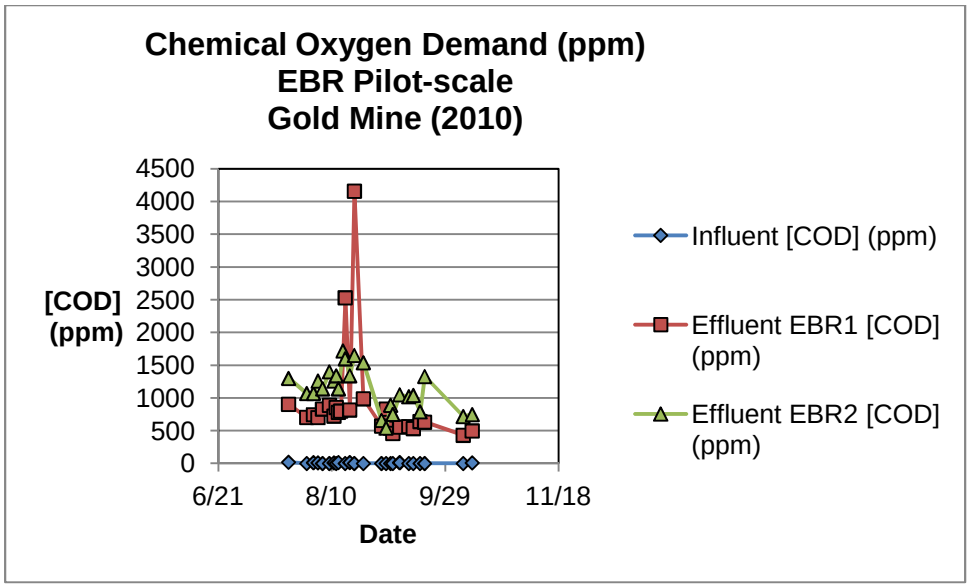

Figure B9. Chemical Oxygen Demand EBR Pilot-Scale (2010).

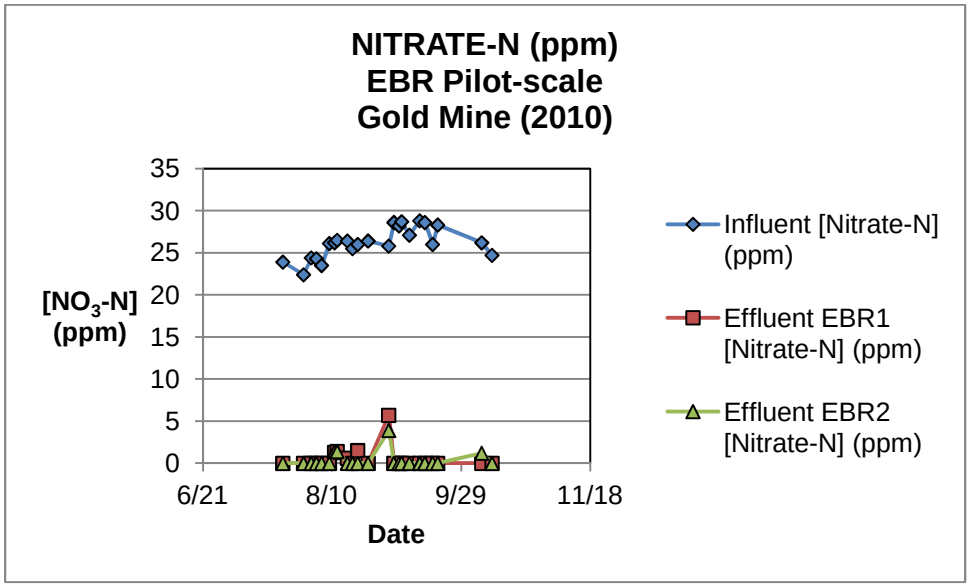

Figure B10. Nitrate-N EBR Pilot-Scale (2010).

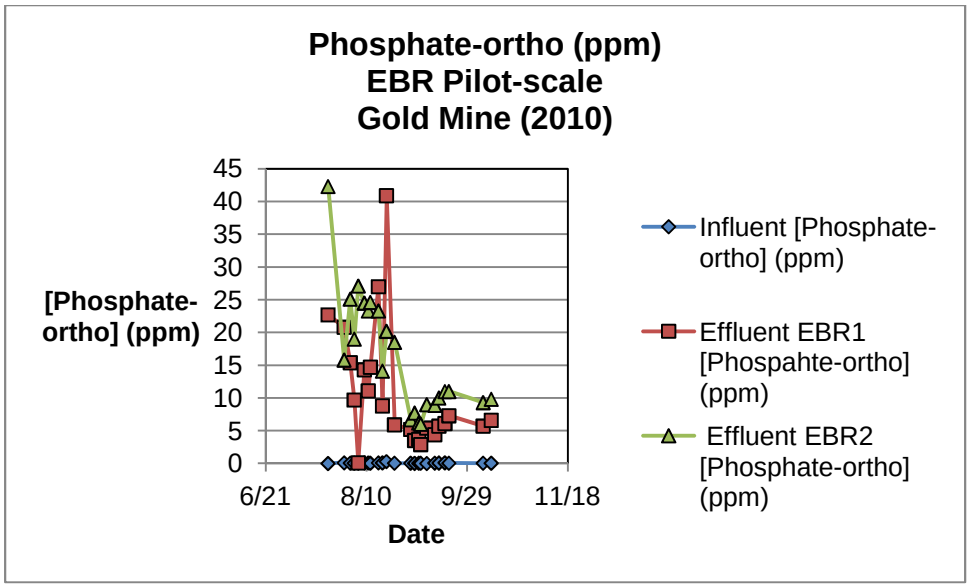

Figure B11. Phosphate-ortho EBR Pilot-Scale (2010).

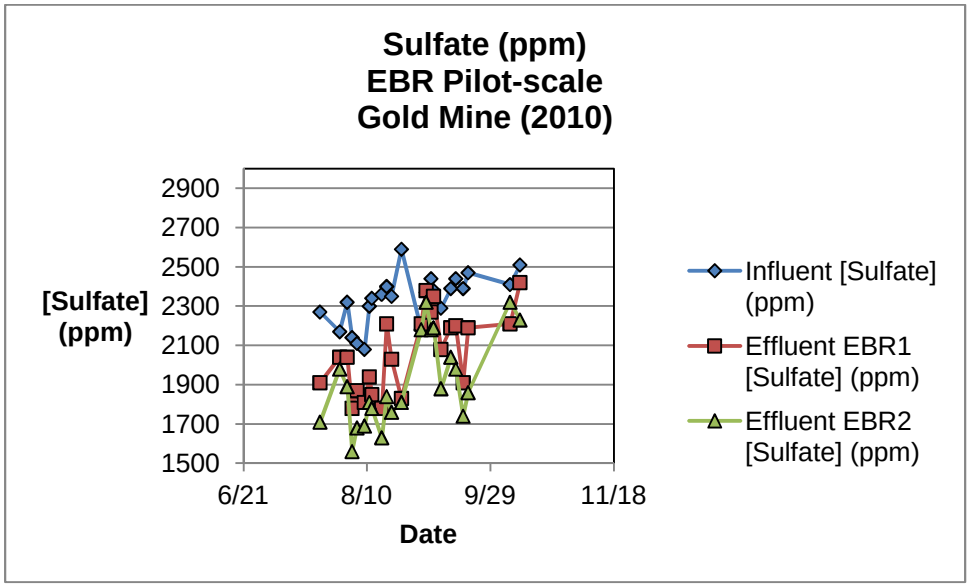

Figure B12. Sulfate EBR Pilot-Scale (2010).

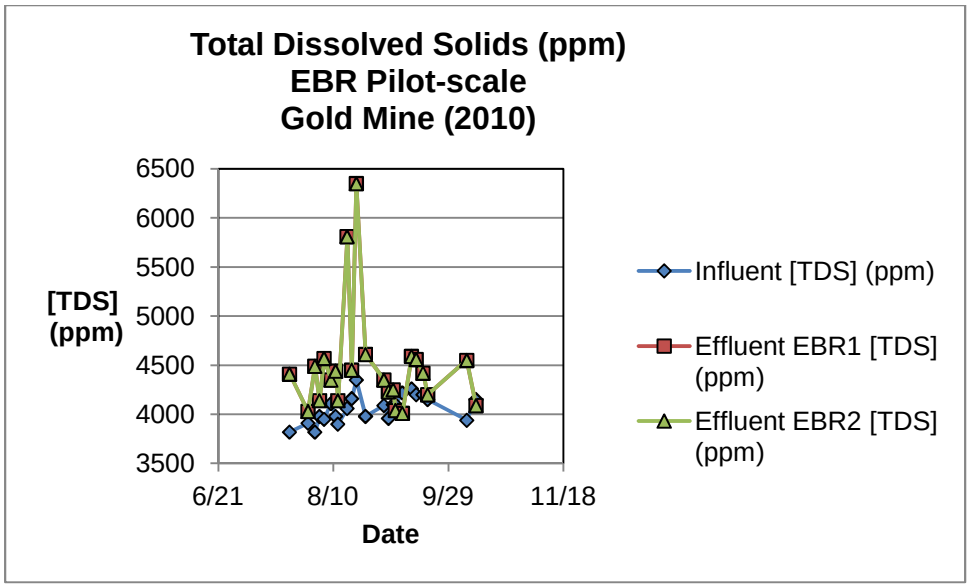

Figure B13. Total Dissolved Solids EBR Pilot-Scale (2010).

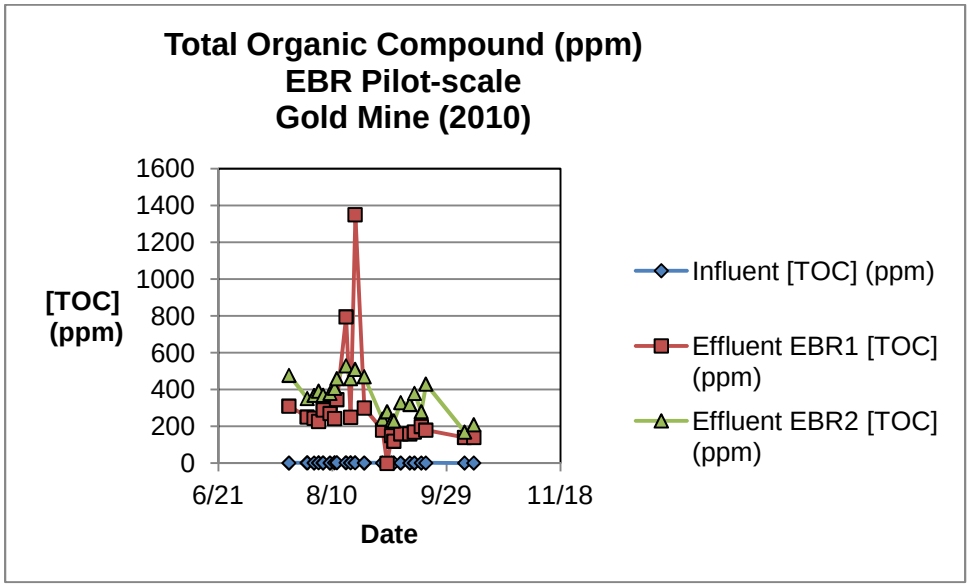

Figure B14. Total Organic Compound EBR Pilot-Scale (2010).

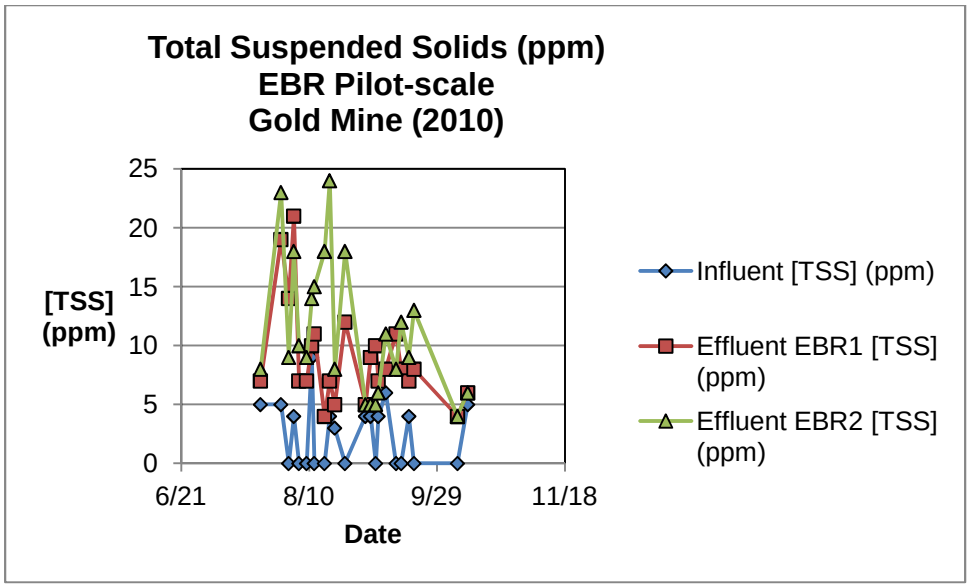

Figure B15. Total Suspended Solids EBR Pilot-Scale (2010).

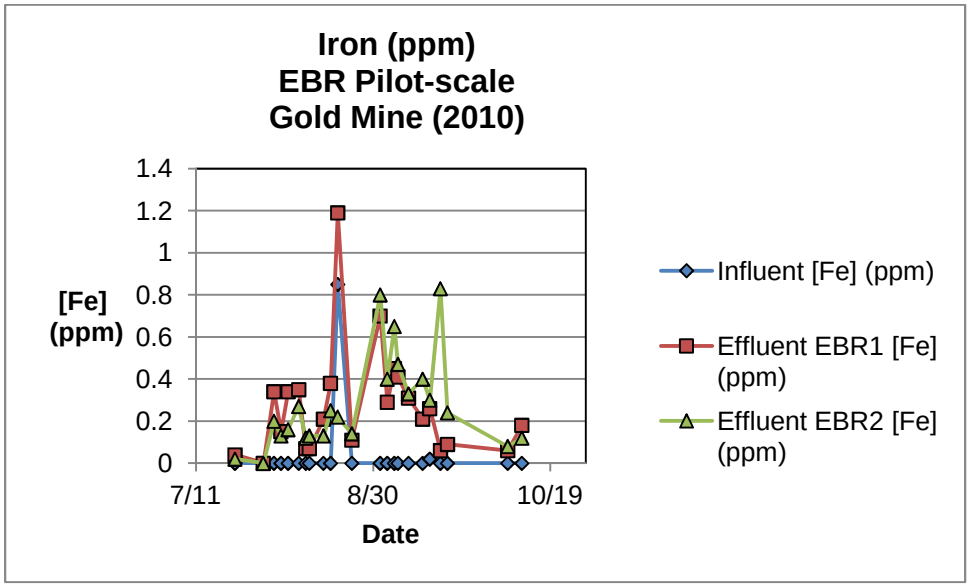

Figure B16. Iron EBR Pilot-Scale (2010).

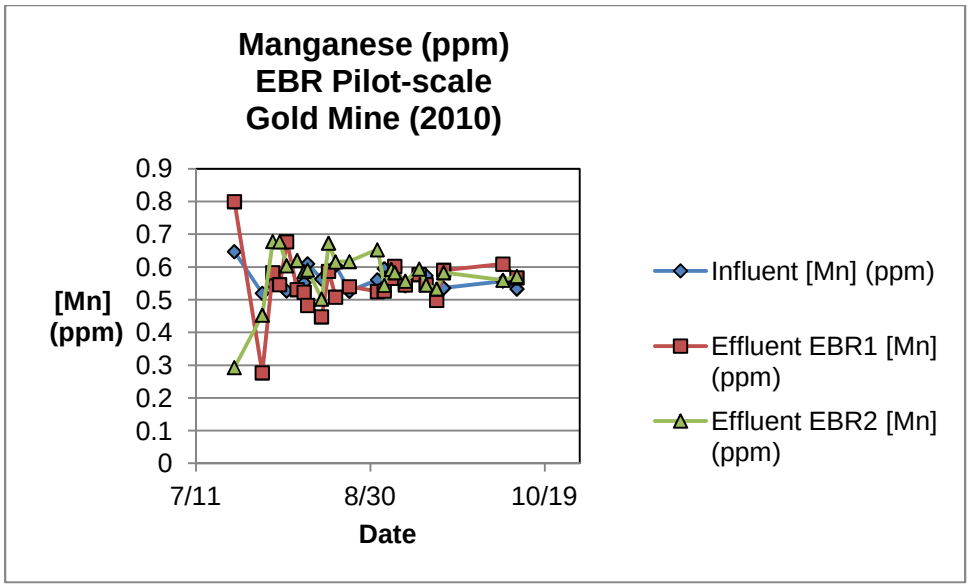

Figure B17. Manganese EBR Pilot-Scale (2010).

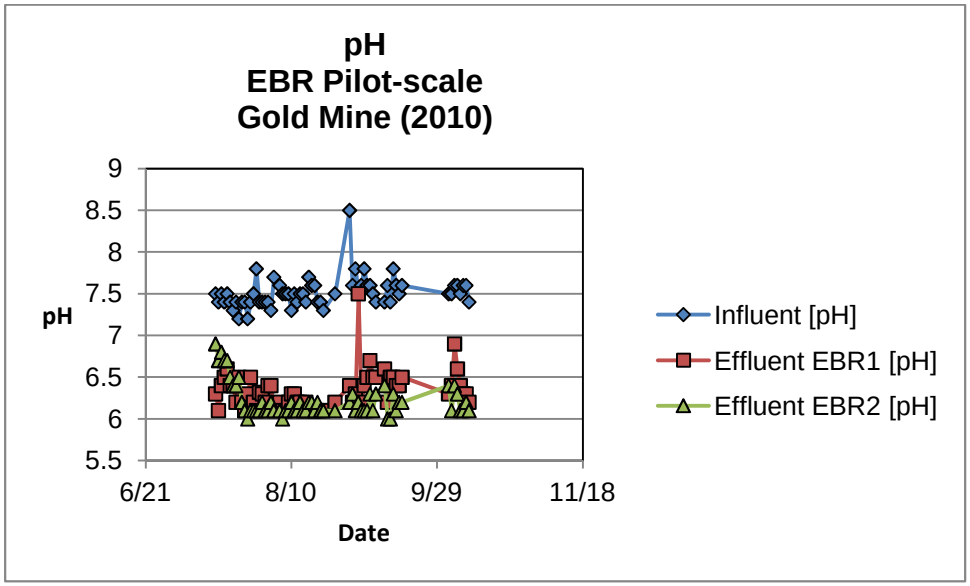

Figure B18. pH EBR Pilot-Scale (2010).

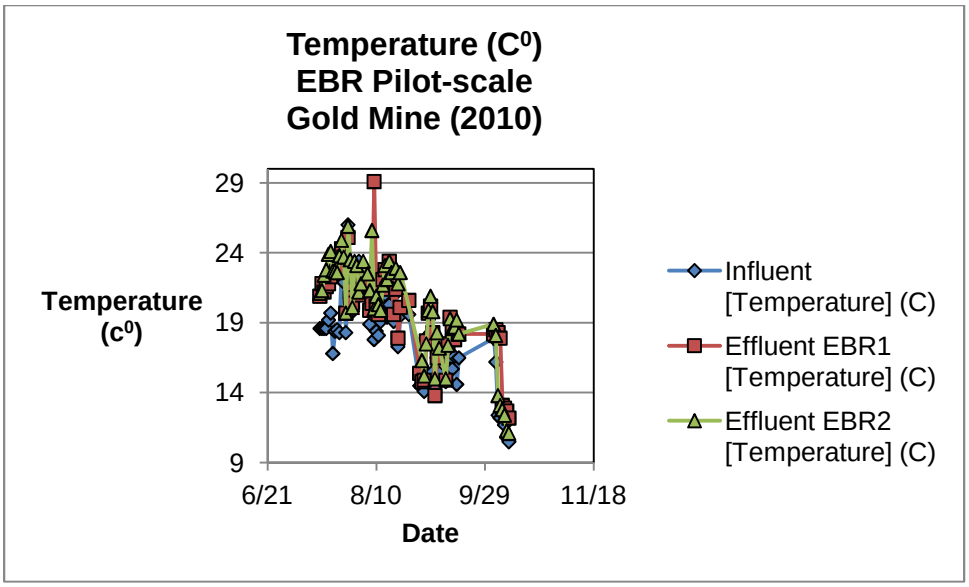

Figure B19. Temperature EBR Pilot-Scale (2010).

APPENDIX C

DATA AND GRAPHS COLLECTED FROM EBR BENCH-SCALE TESTS

FROM BASED METAL MINE WATERS

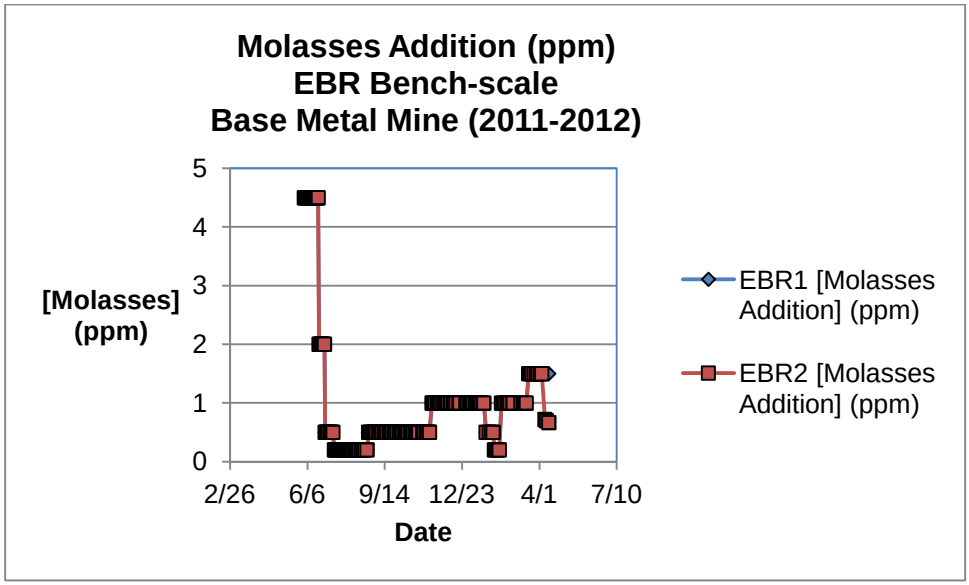

Figure C1. Molasses addition EBR Bench-Scale (2011-2012).

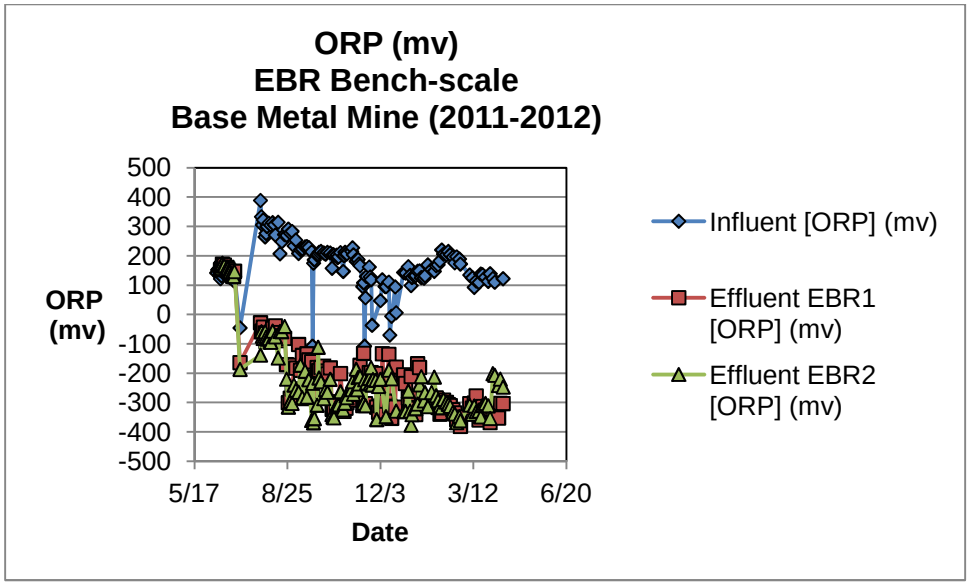

Figure C2. ORP EBR Bench-Scale (2011-2012).

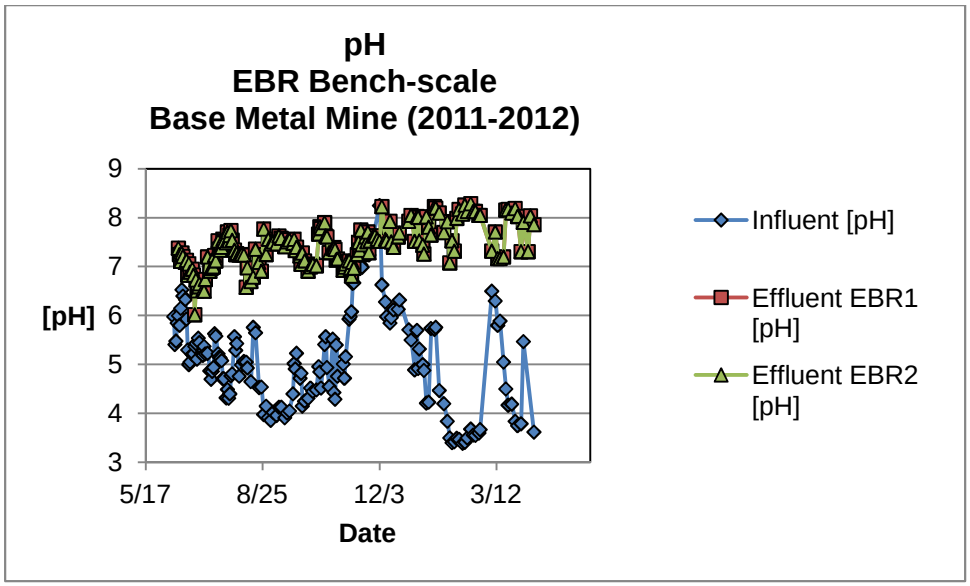

Figure C3. pH EBR Bench-Scale (2011-2012).

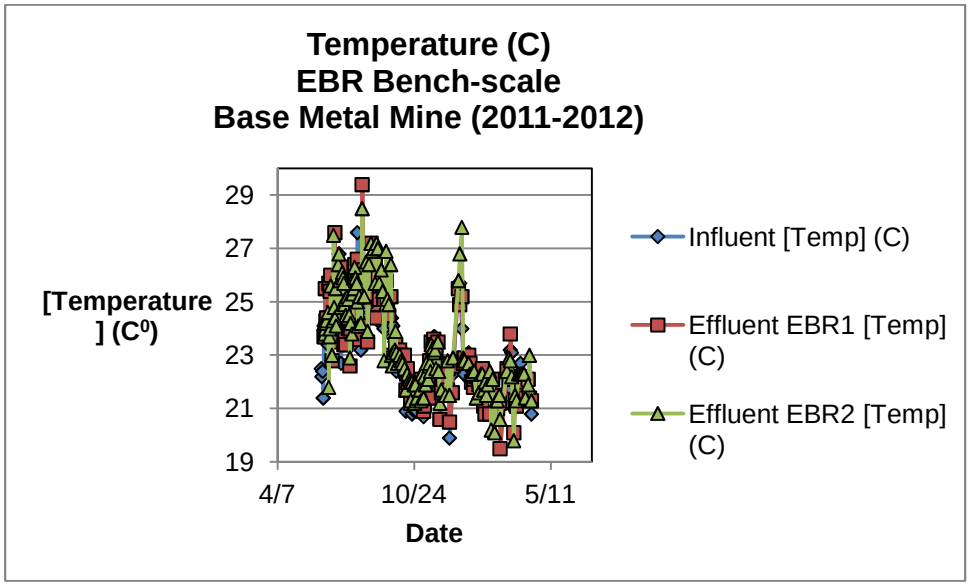

Figure C4. Temperature EBR Bench-Scale (2011-2012).

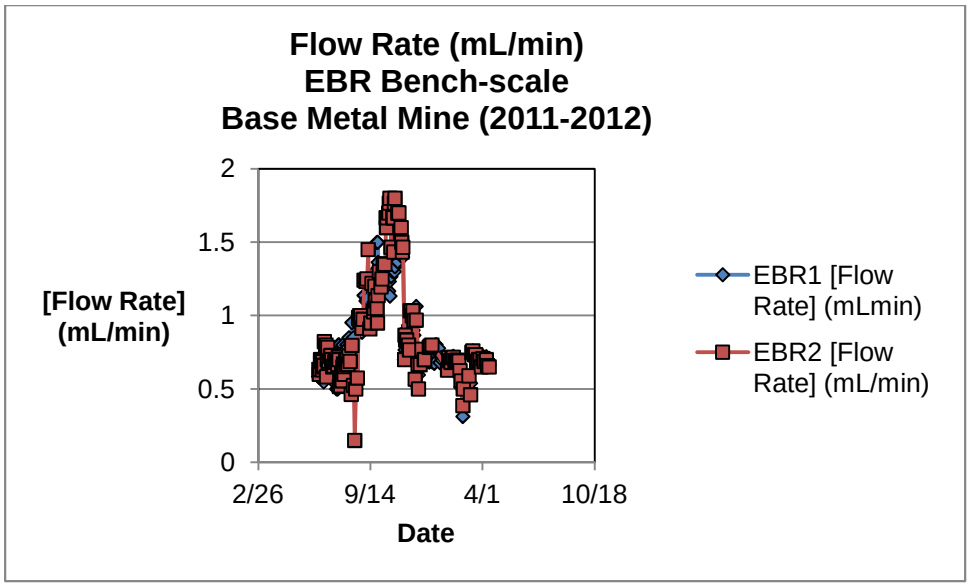

Figure C5. Flow Rate EBR Bench-Scale (2011-2012).

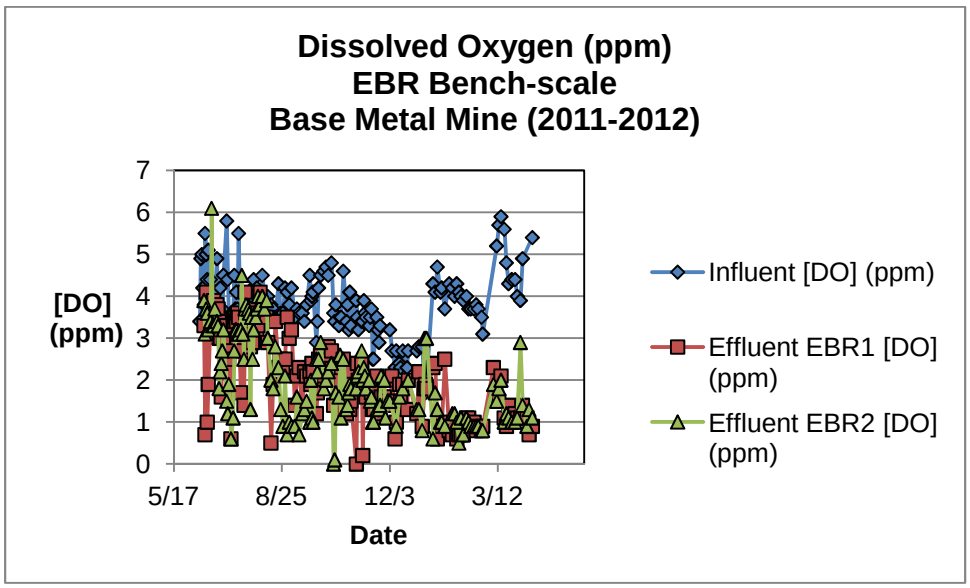

Figure C6. Dissolved Oxygen EBR Bench-Scale (2011-2012).

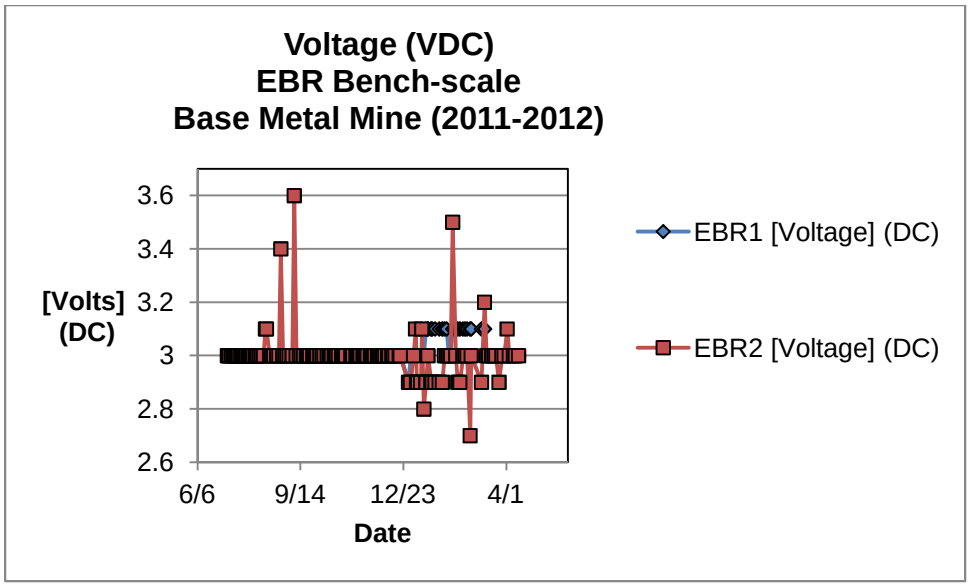

Figure C7. Voltage EBR Bench-Scale (2011-2012).

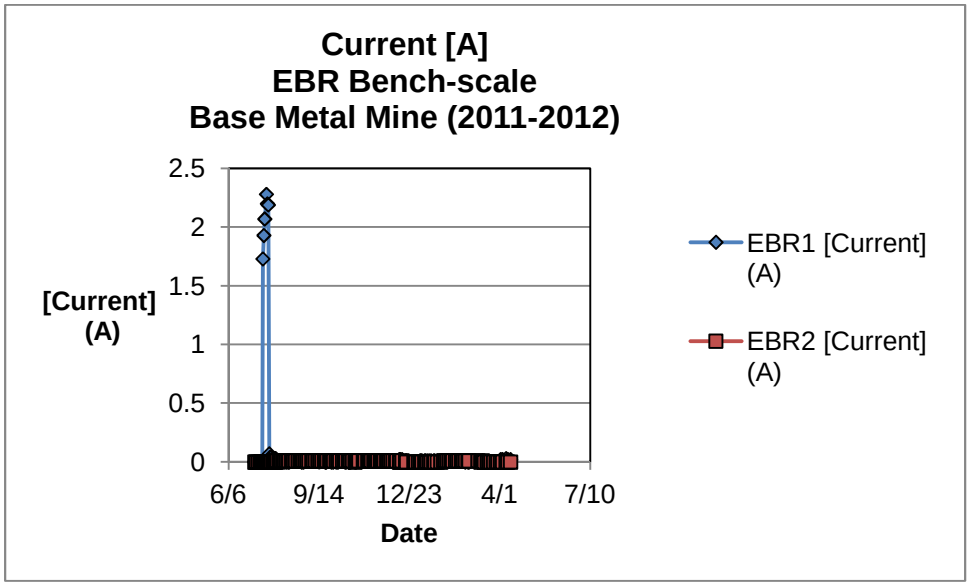

Figure C8. Current EBR Bench-Scale (2011-2012).

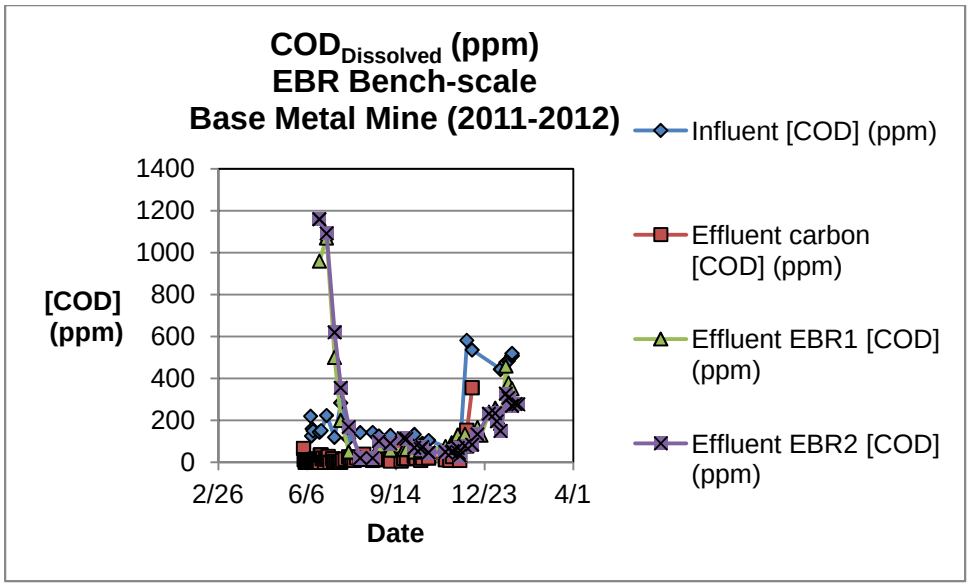

Figure C9. $COD_{Dissolved}$ EBR Bench-Scale (2011-2012).

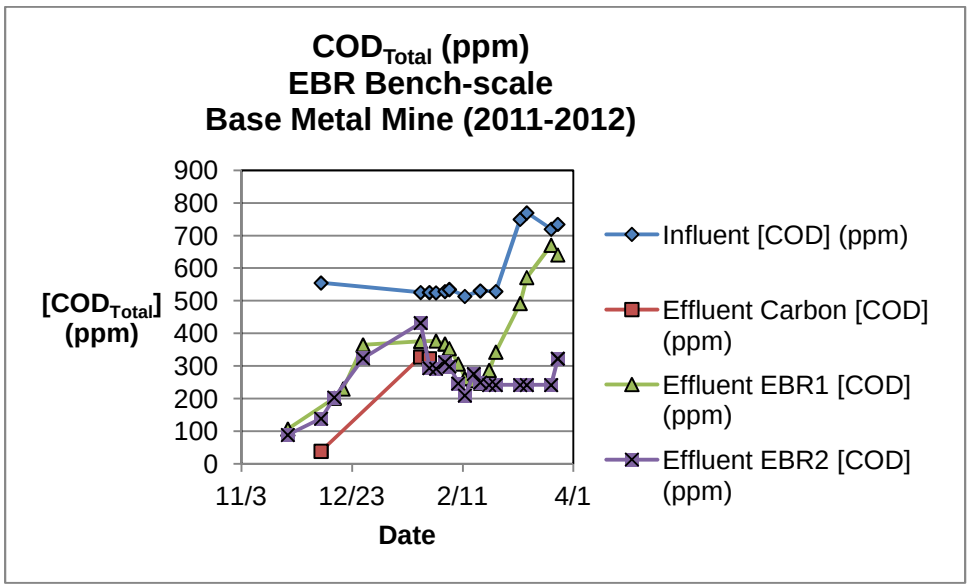

Figure C10. COD_{Total} EBR Bench-Scale (2011-2012).

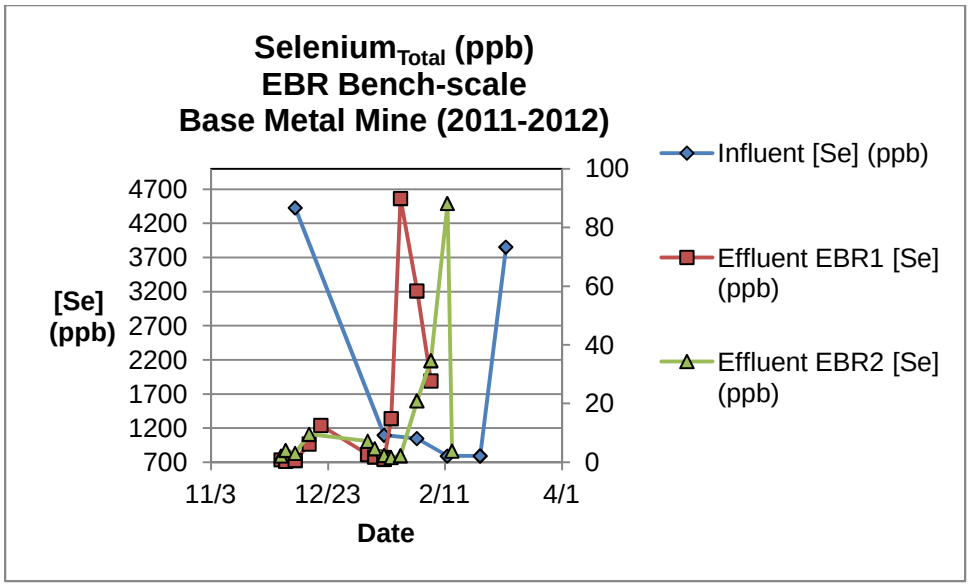

Figure C11. Selenium$_{Total}$ EBR Bench-Scale (2011-2012).

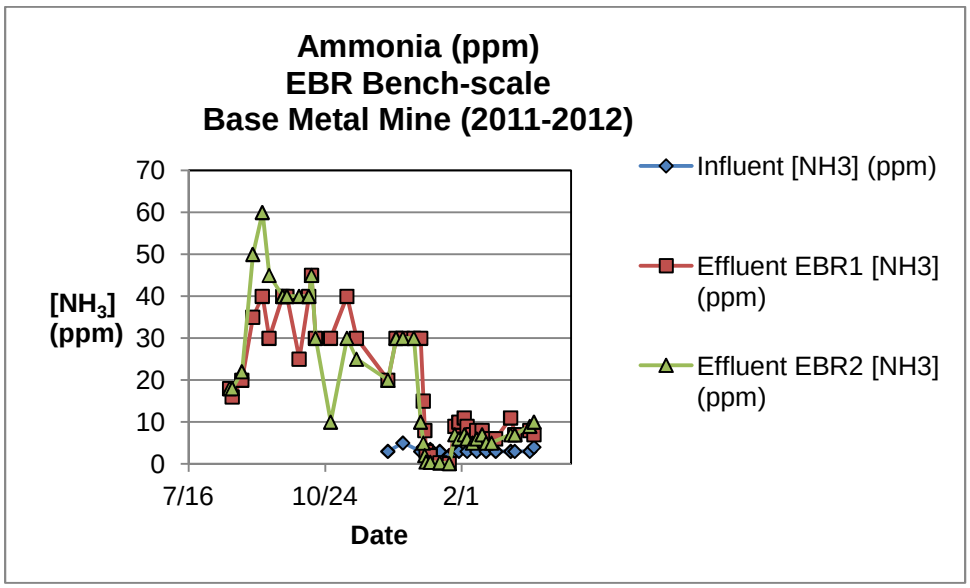

Figure C12. Ammonia EBR Bench-Scale (2011-2012).

APPENDIX D

DATA AND GRAPHS FROM EBR PILOT-SCALE TESTING OF BASE METAL MINE WATERS

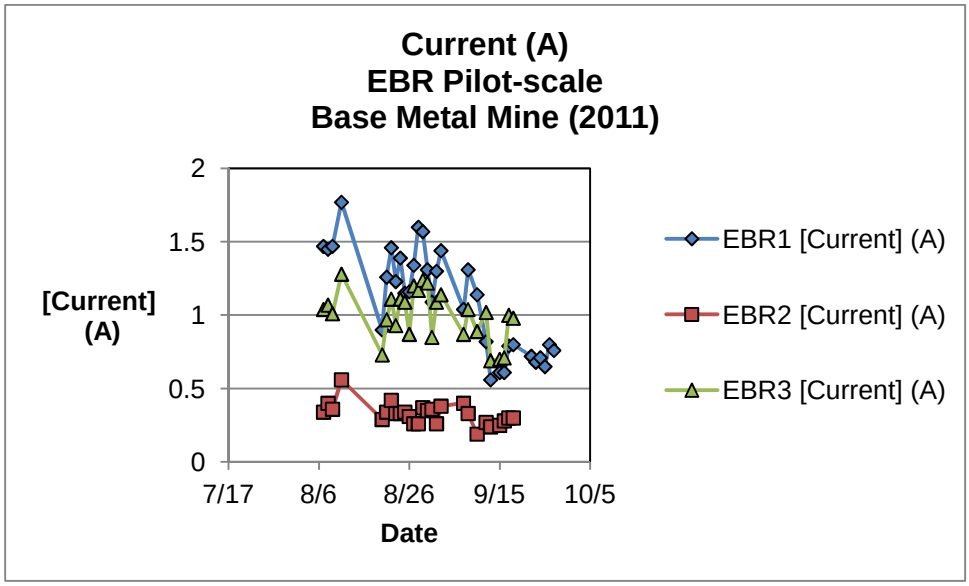

Figure D1. Current EBR Pilot-Scale (2011).

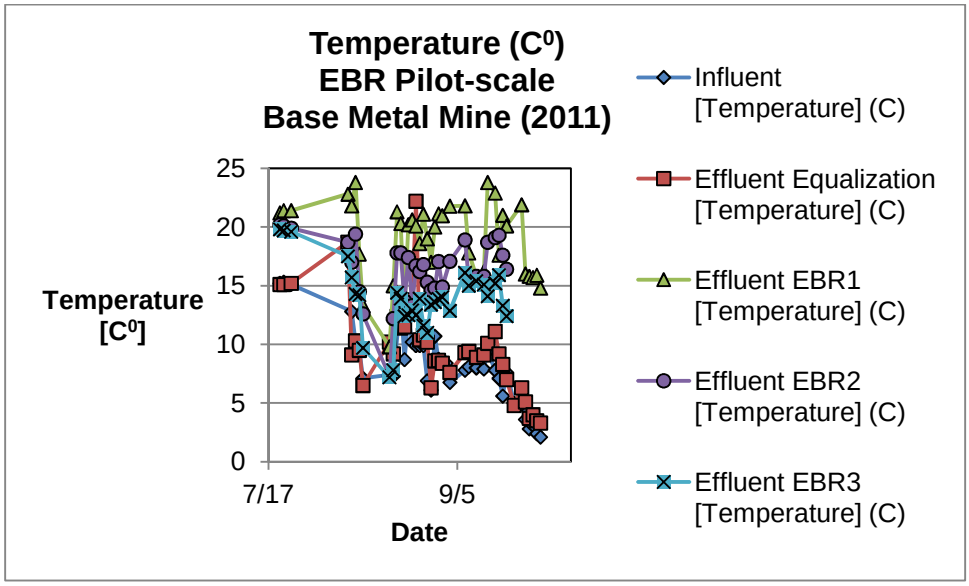

Figure D2. Temperature EBR Pilot-Scale (2011).

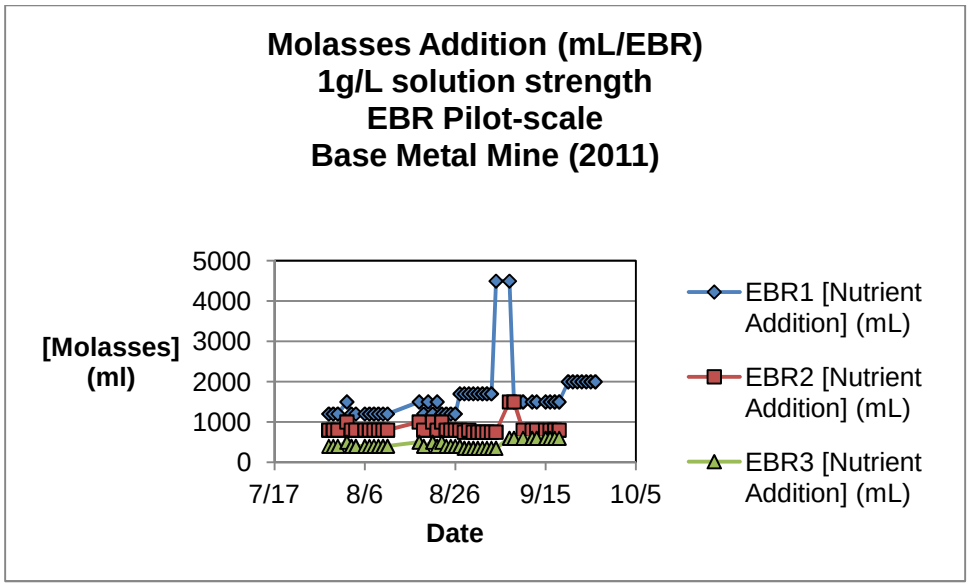

Figure D3. Molasses Addition EBR Pilot-Scale (2011).

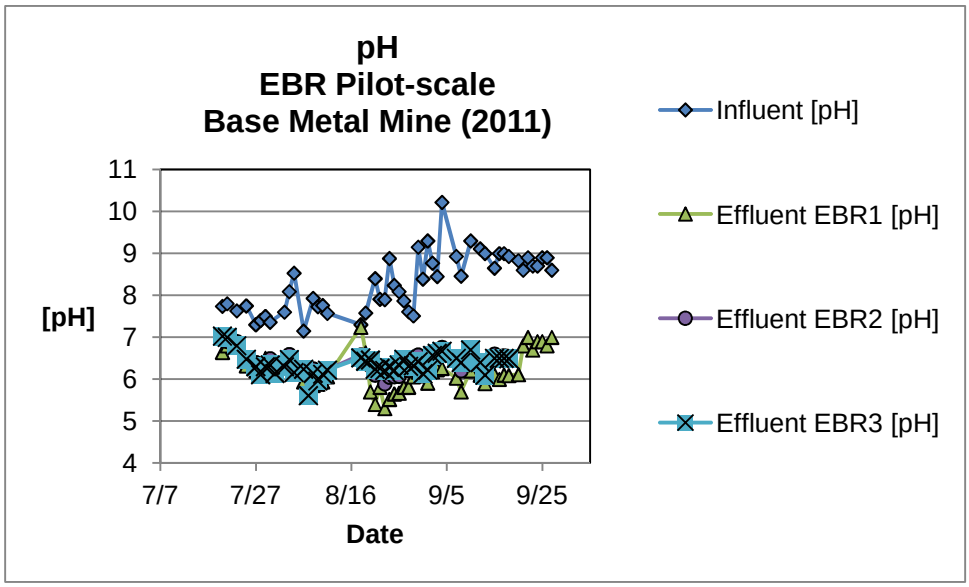

Figure D4. pH EBR Pilot-Scale (2011).

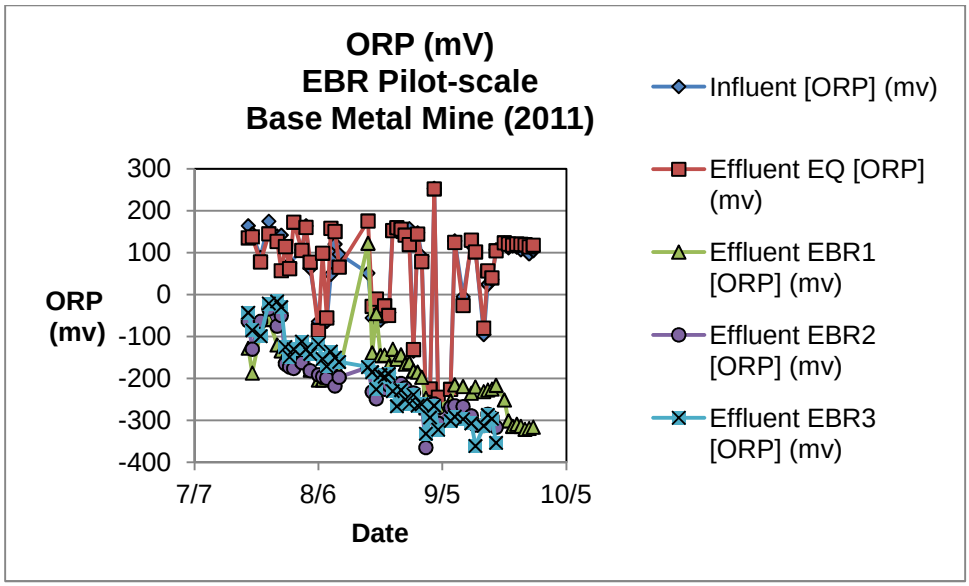

Figure D5. ORP EBR Pilot-Scale (2011).

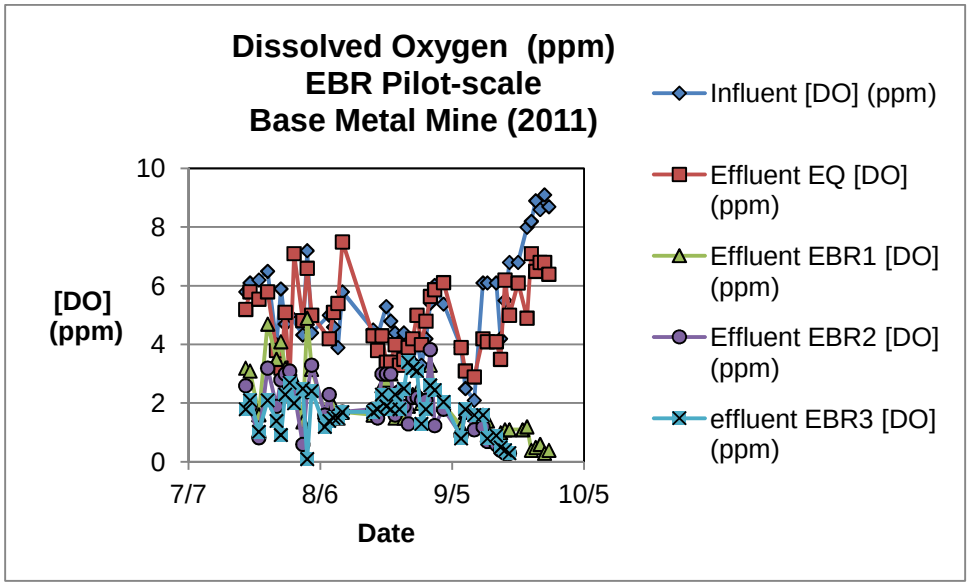

Figure D6. Dissolved Oxygen EBR Pilot-Scale (2011).

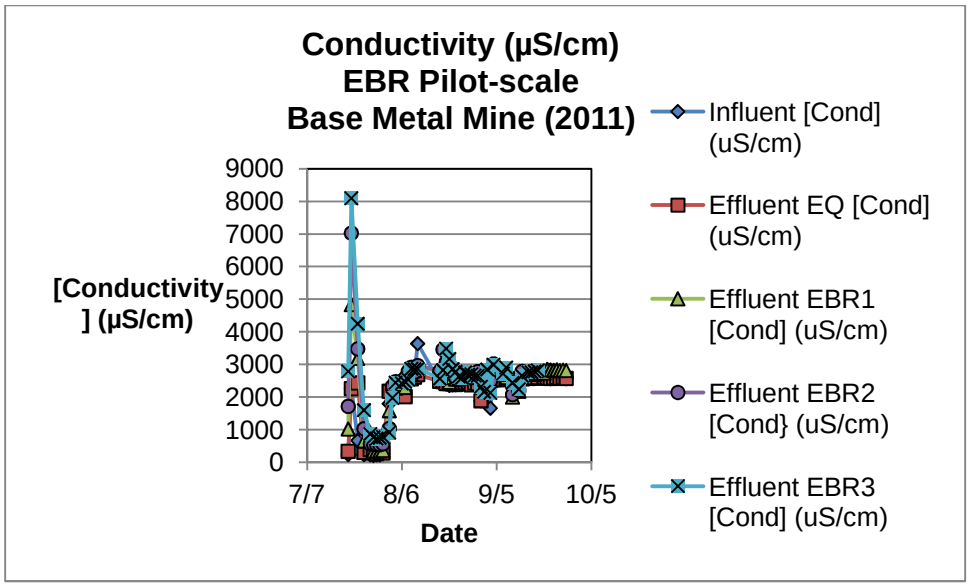

Figure D7. Conductivity EBR Pilot-Scale (2011).

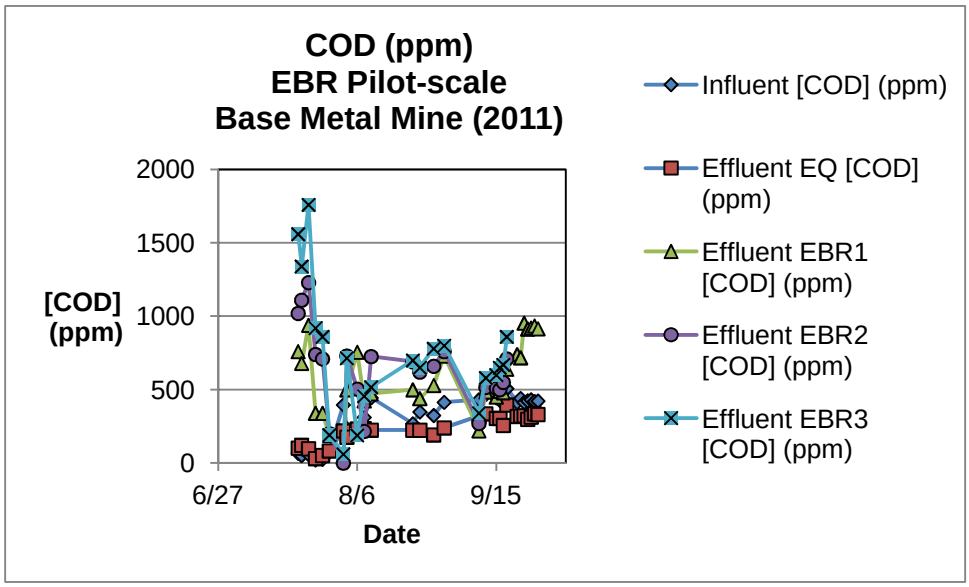

Figure D8. COD EBR Pilot-Scale (2011).

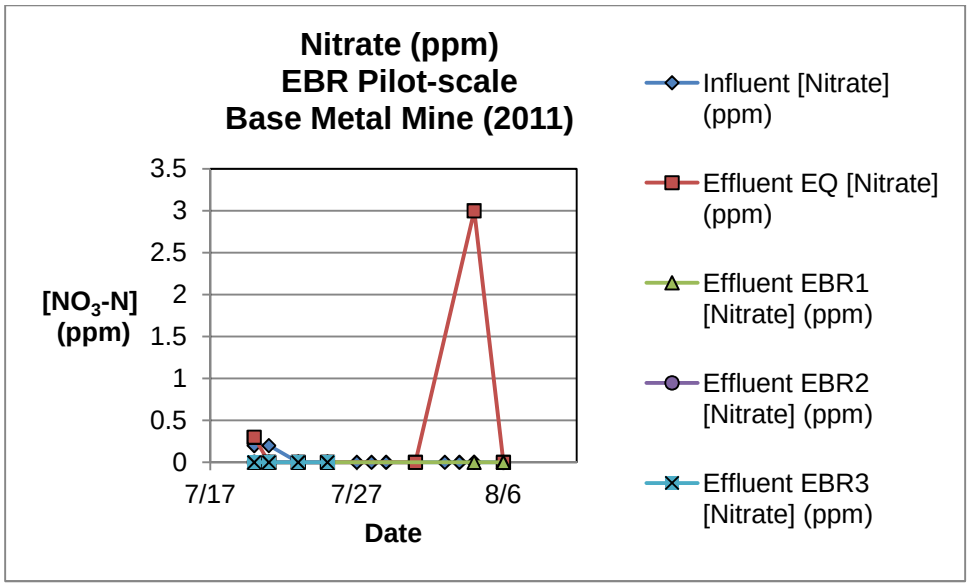

Figure D9. Nitrate EBR Pilot-Scale (2011).

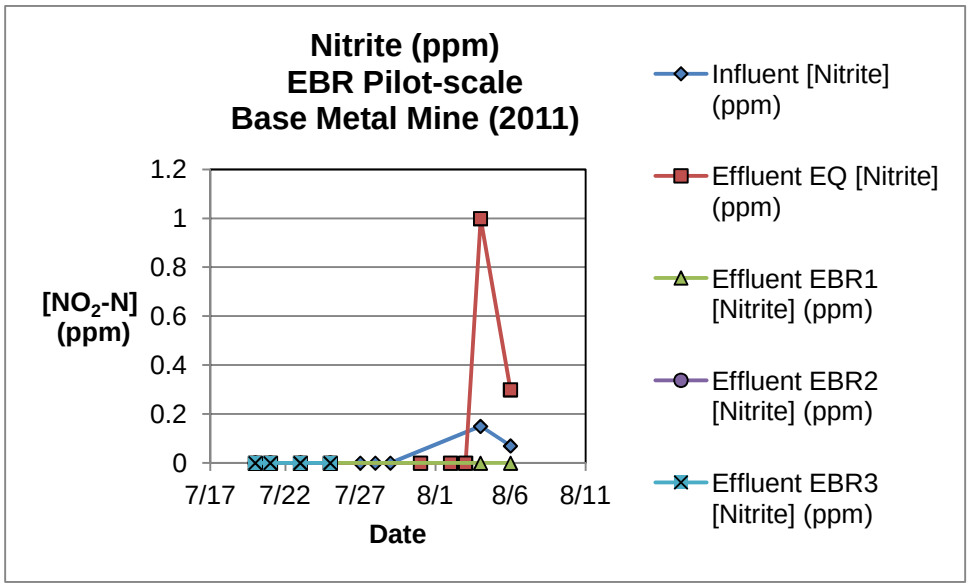

Figure D10. Nitrite EBR Pilot-Scale (2011).

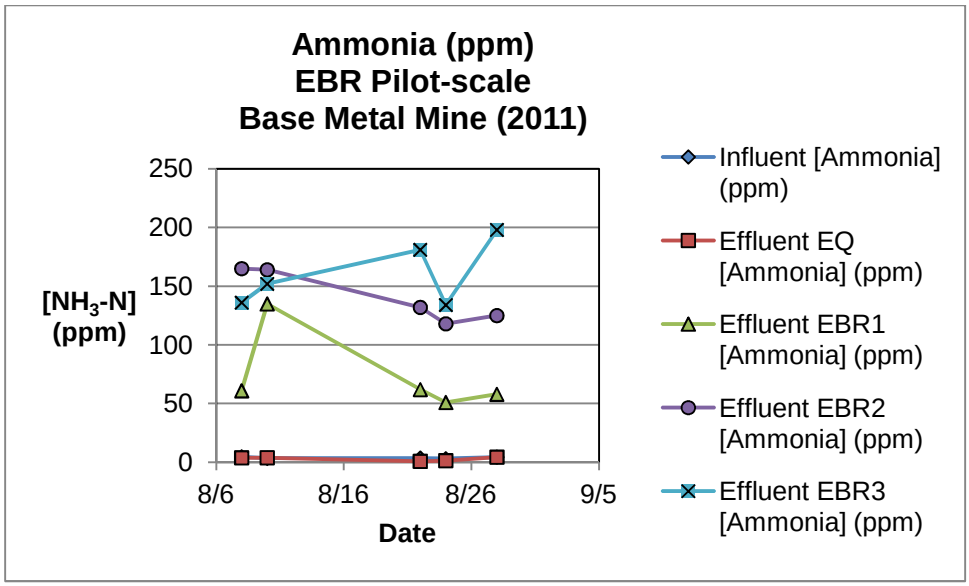

Figure D11. Ammonia EBR Pilot-Scale (2011).

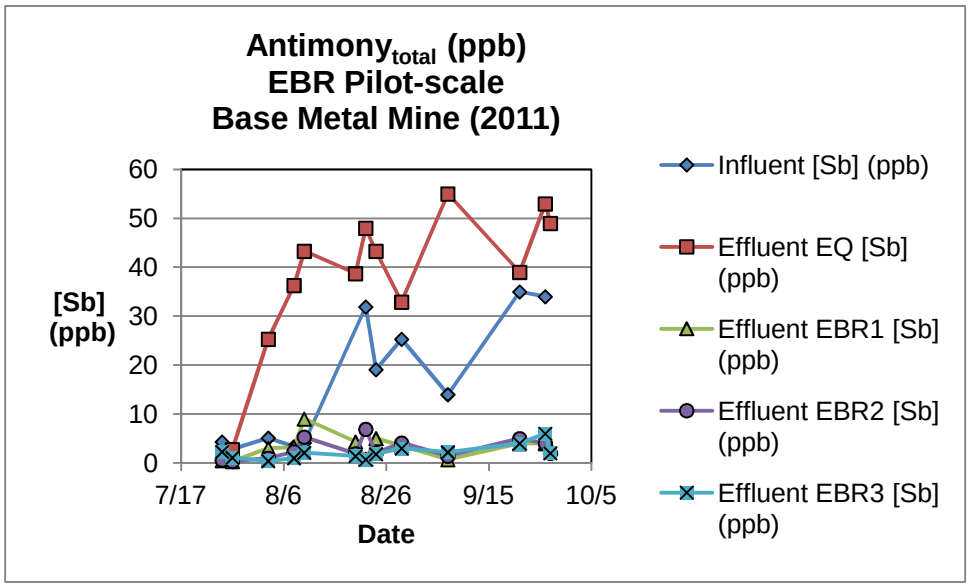

Figure D12. Antimony$_{Total}$ EBR Pilot-Scale (2011).

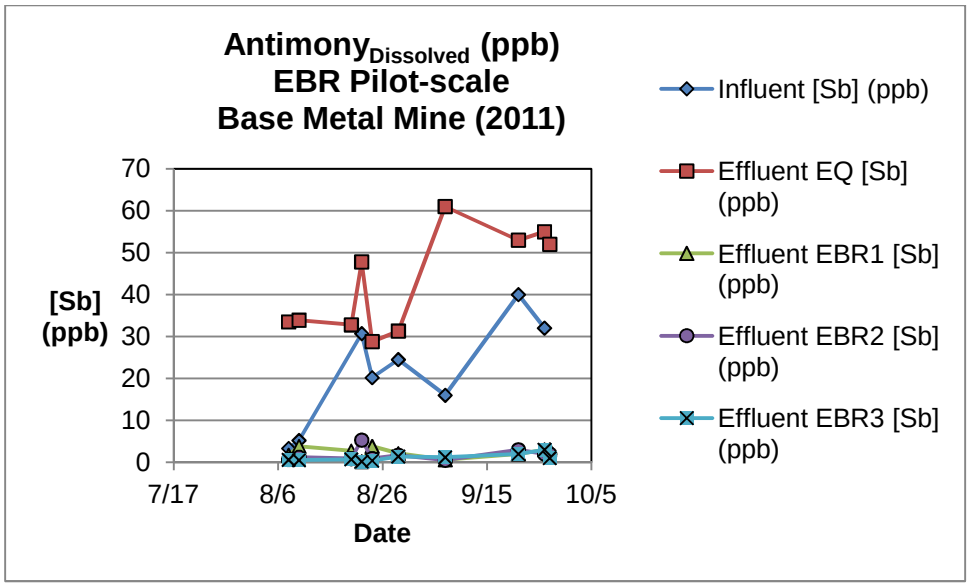

Figure D13. Antimony$_{Dissolved}$ EBR Pilot-Scale (2011).

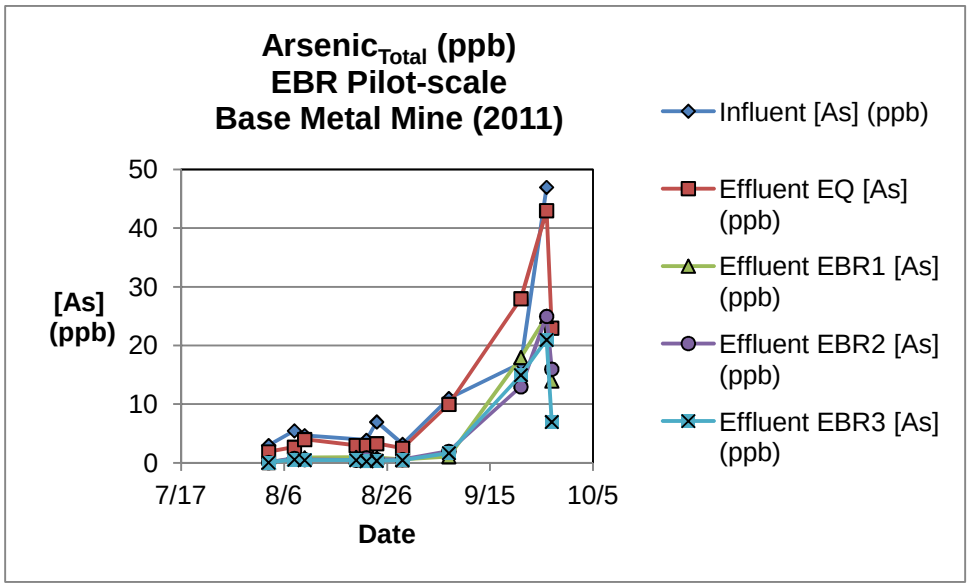

Figure D14. Arsenic$_{Total}$ EBR Pilot-Scale (2011).

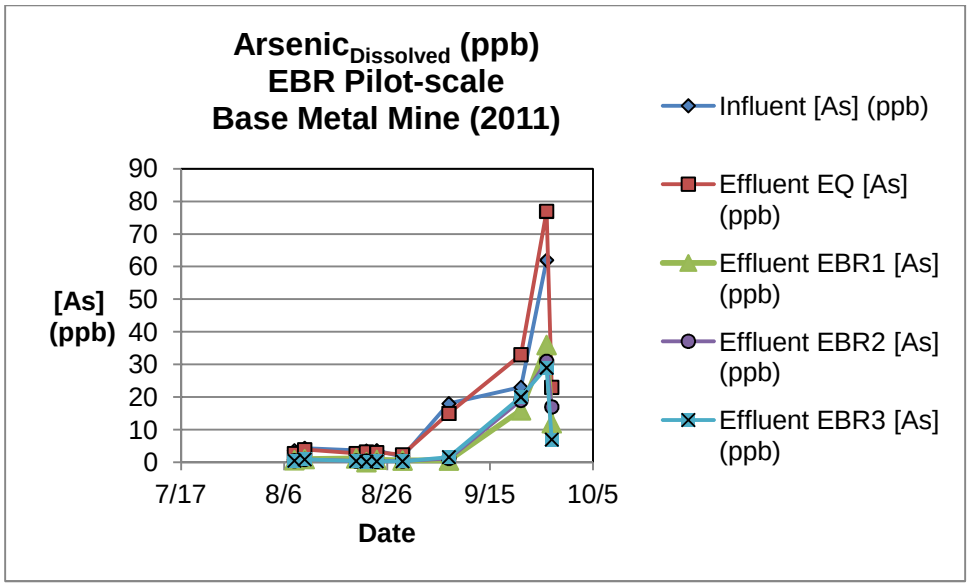

Figure D15. Arsenic$_{Dissolved}$ EBR Pilot-Scale (2011).

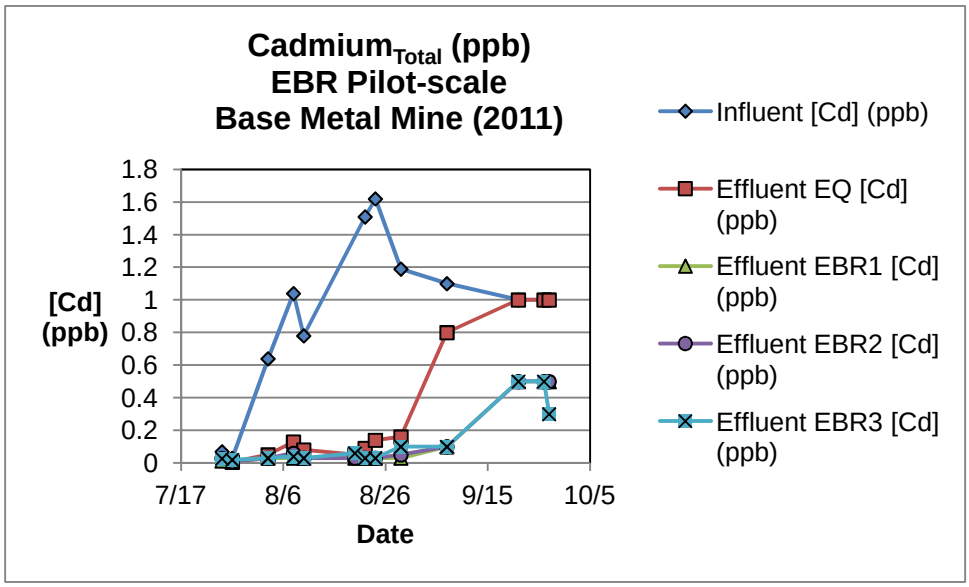

Figure D16. Cadmium$_{Total}$ EBR Pilot-Scale (2011).

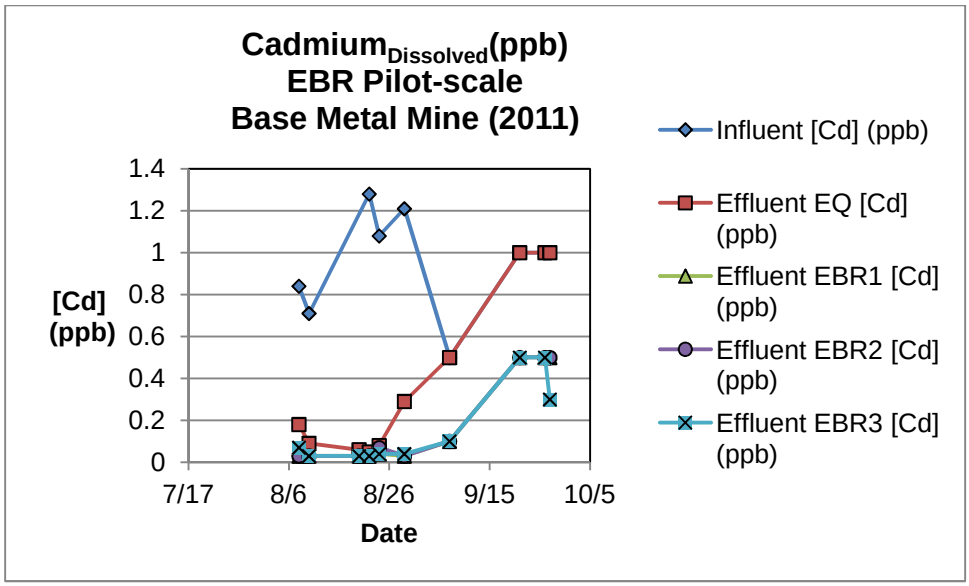

Figure D17. Cadmium$_{Dissolved}$ EBR Pilot-Scale (2011).

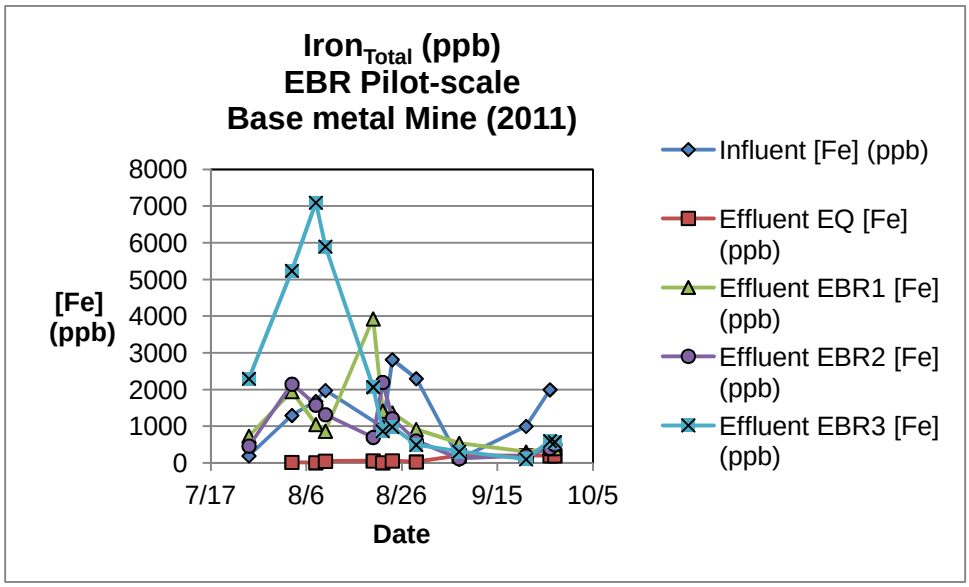

Figure D18. Iron$_{Total}$ EBR Pilot-Scale (2011).

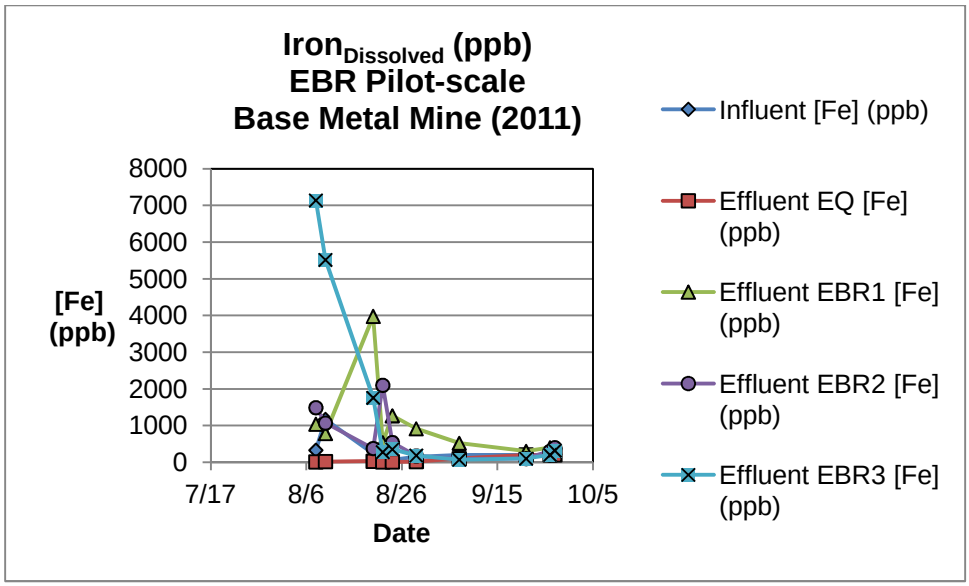

Figure D19. $Iron_{Dissolved}$ EBR Pilot-Scale (2011).

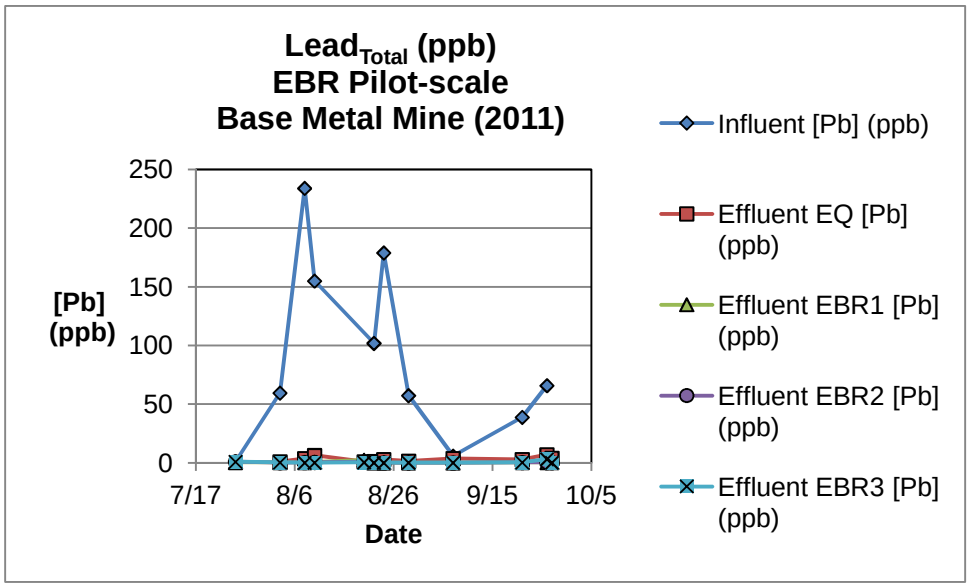

Figure D20. $Lead_{Total}$ EBR Pilot-Scale (2011).

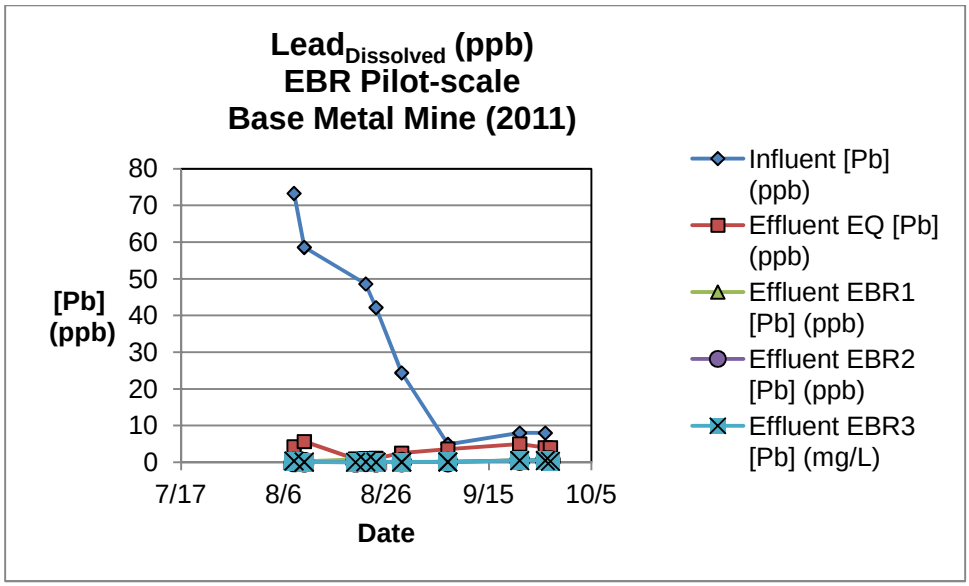

Figure D21. $Lead_{Dissolved}$ EBR Pilot-Scale (2011).

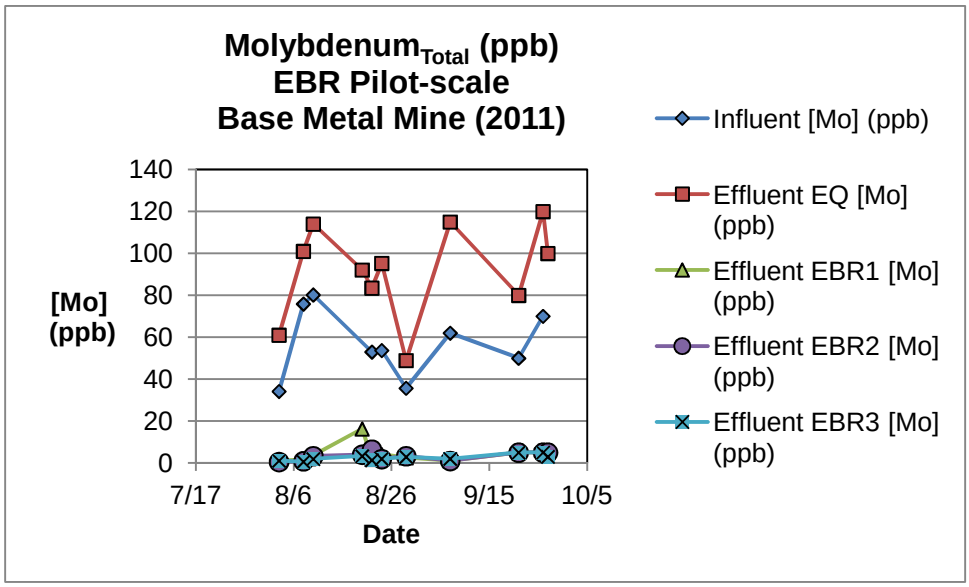

Figure D22. $Molybdenum_{Total}$ EBR Pilot-Scale (2011).

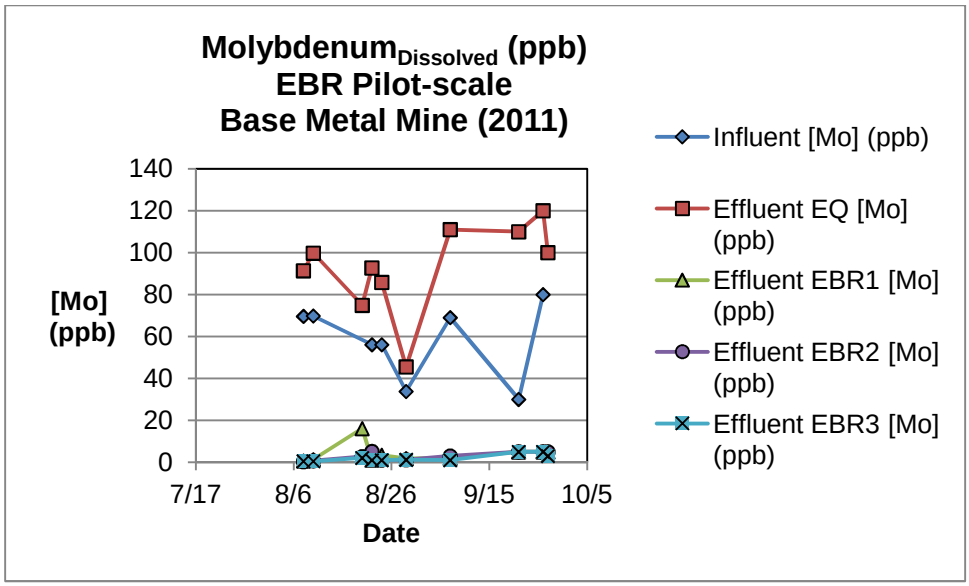

Figure D23. Molybdenum$_{Dissolved}$ EBR Pilot-Scale (2011).

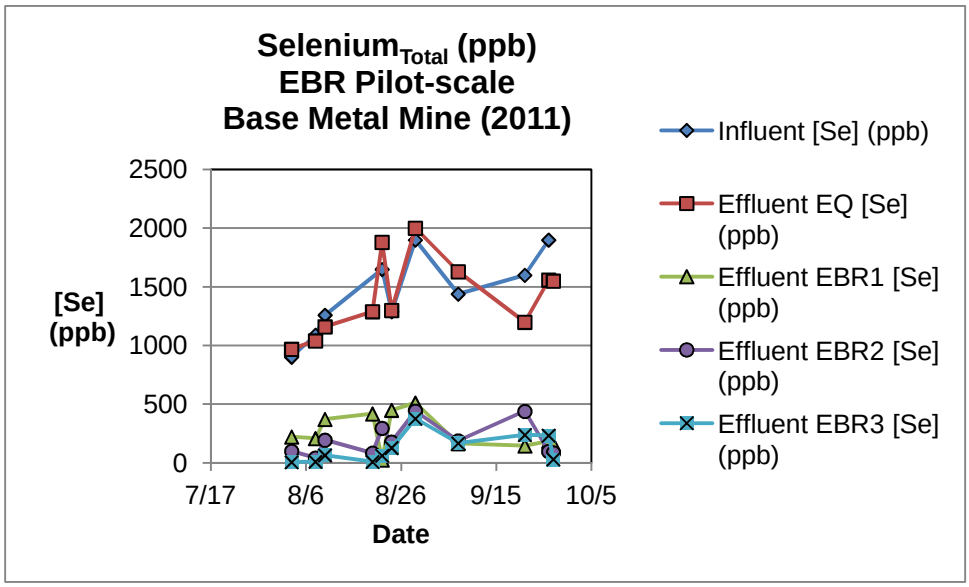

Figure D24. Selenium$_{Total}$ EBR Pilot-Scale (2011).

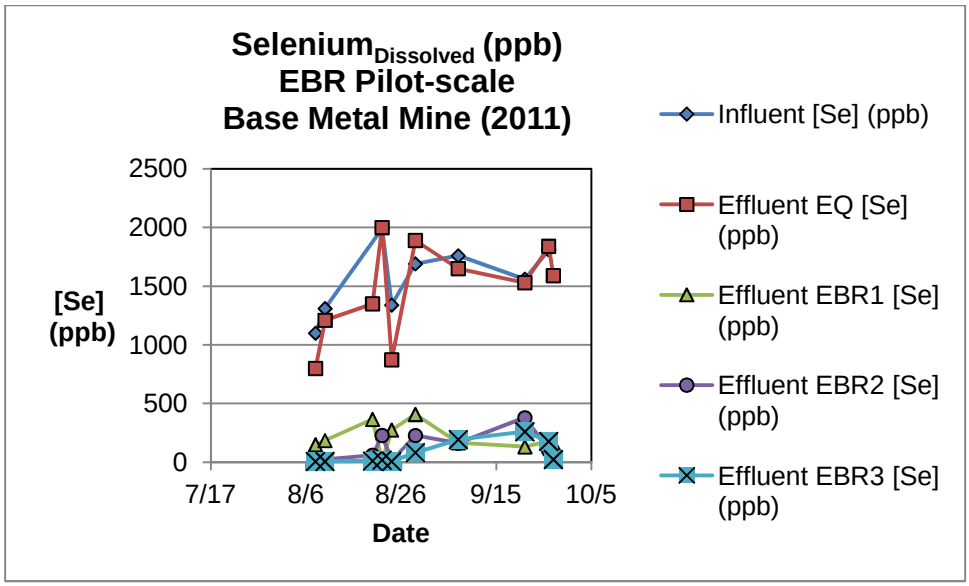

Figure D25. Selenium$_{Dissolved}$ EBR Pilot-Scale (2011).

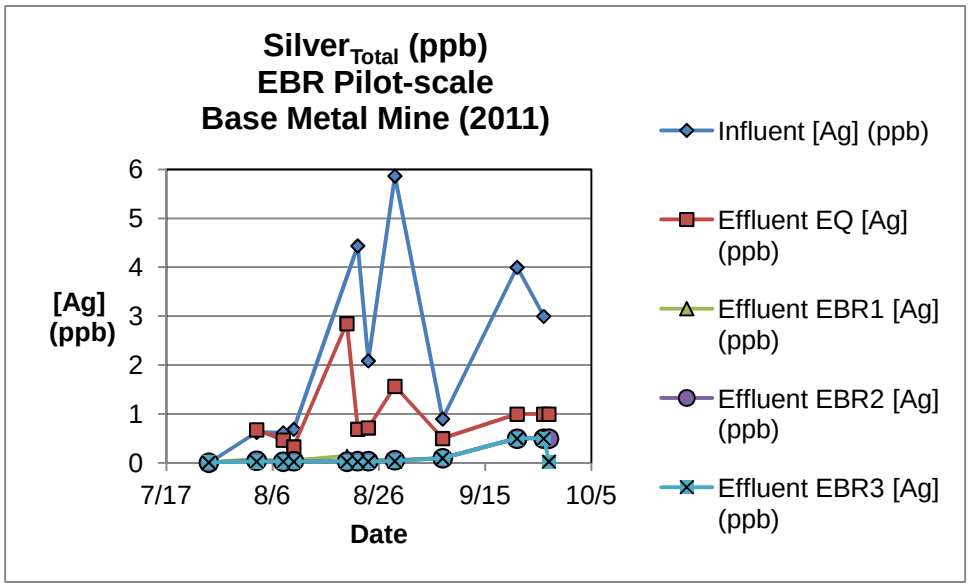

Figure D26. Silver$_{Total}$ EBR Pilot-scale (2011).

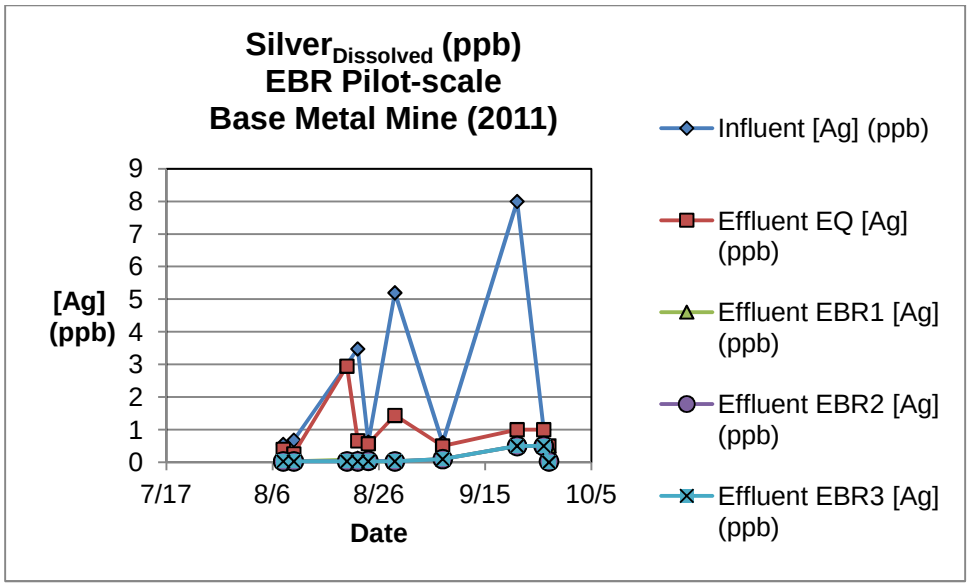

Figure D27. Silver$_{Dissolved}$ EBR Pilot-Scale (2011).

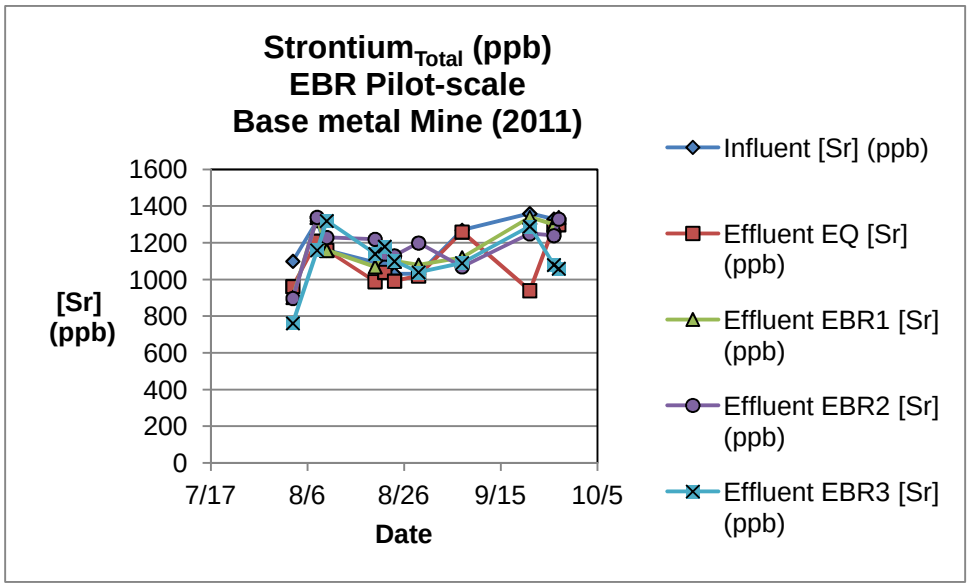

Figure D28. Strontium$_{Total}$ EBR Pilot-Scale (2011).

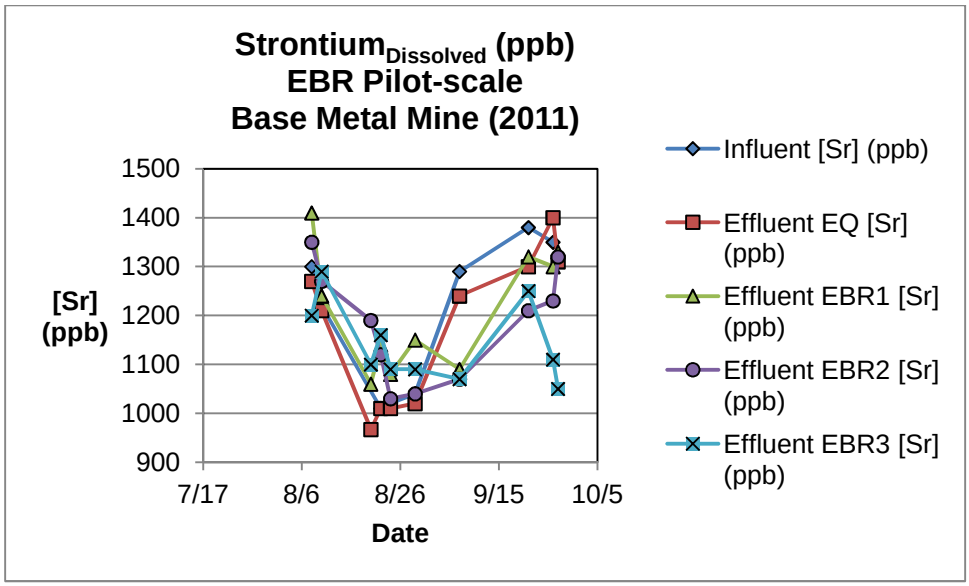

Figure D29. Strontium$_{Dissolved}$ EBR Pilot-Scale (2011).

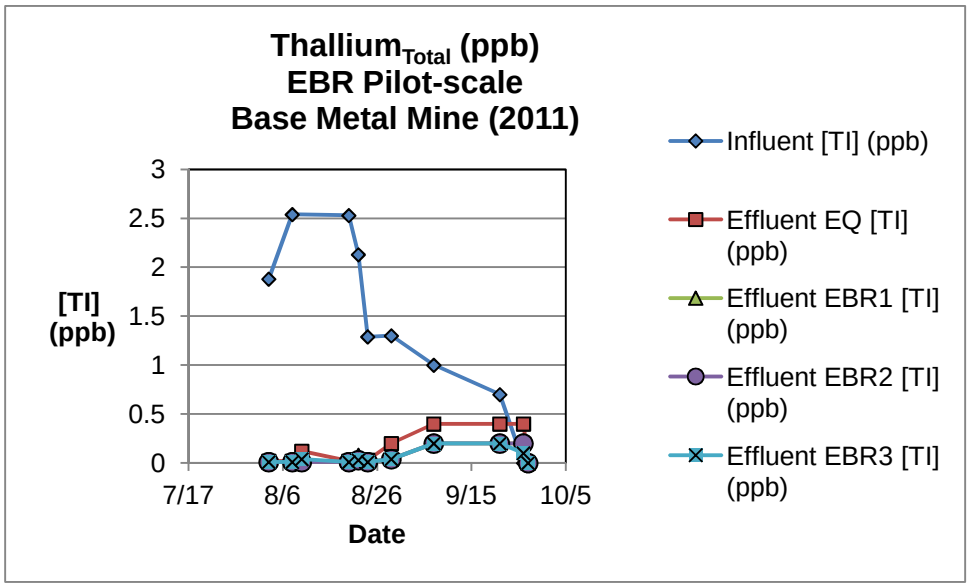

Figure D30. Thallium$_{Total}$ EBR Pilot-Scale (2011).

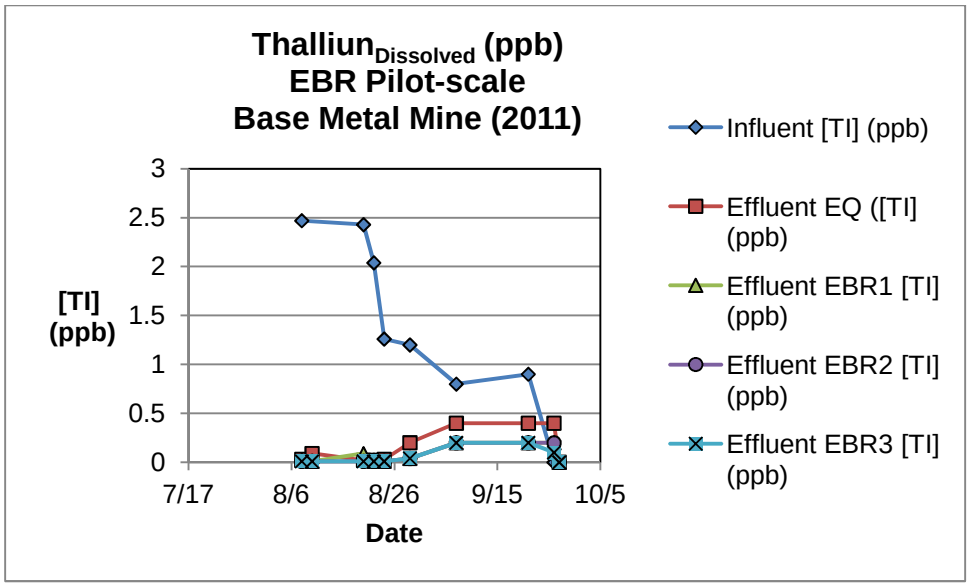

Figure D31. Thallium$_{Dissolved}$ EBR Pilot-Scale (2011).

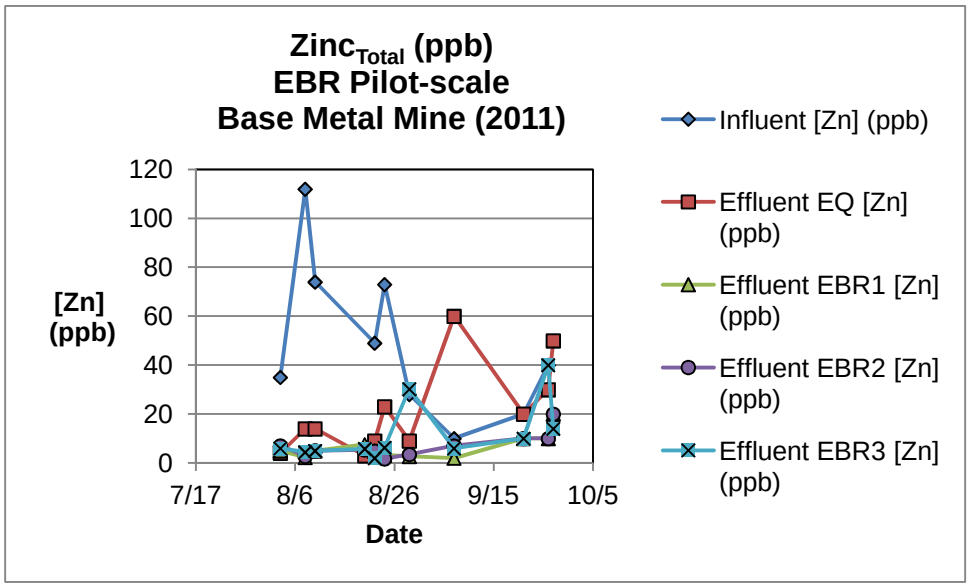

Figure D32. Zinc$_{Total}$ EBR Pilot-Scale (2011).

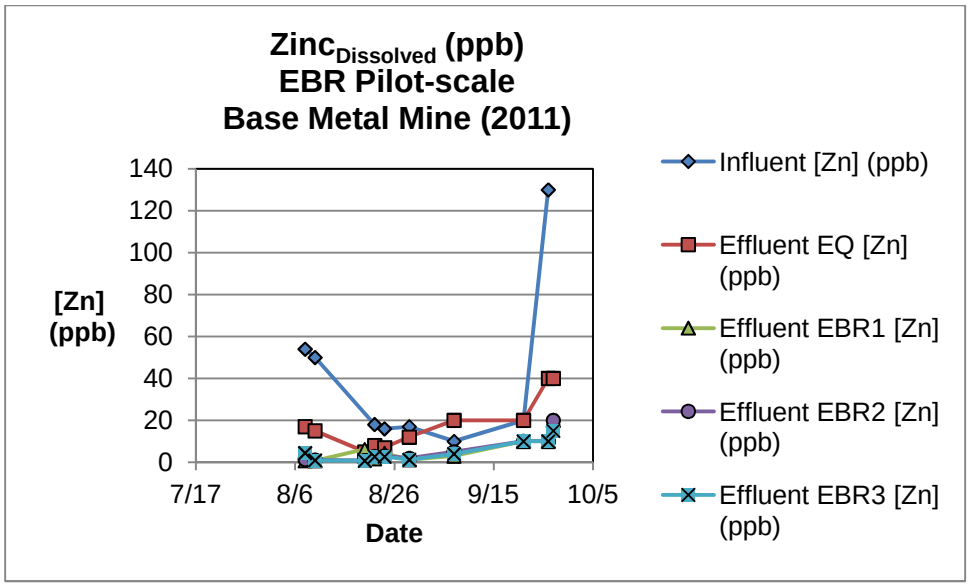

Figure D33. $Zinc_{Dissolved}$ EBR Pilot-Scale (2011).

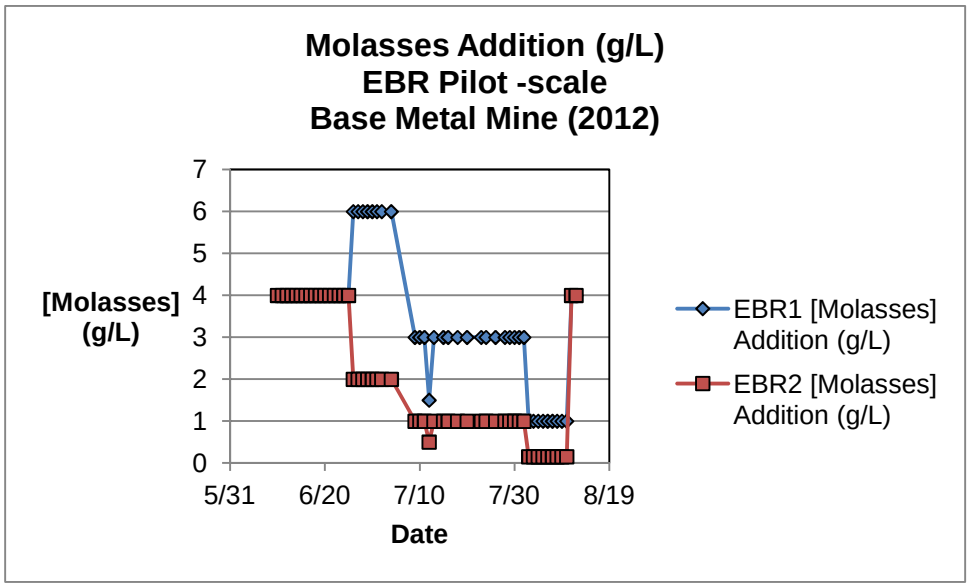

Figure D34. Molasses Addition EBR Pilot-Scale (2012).

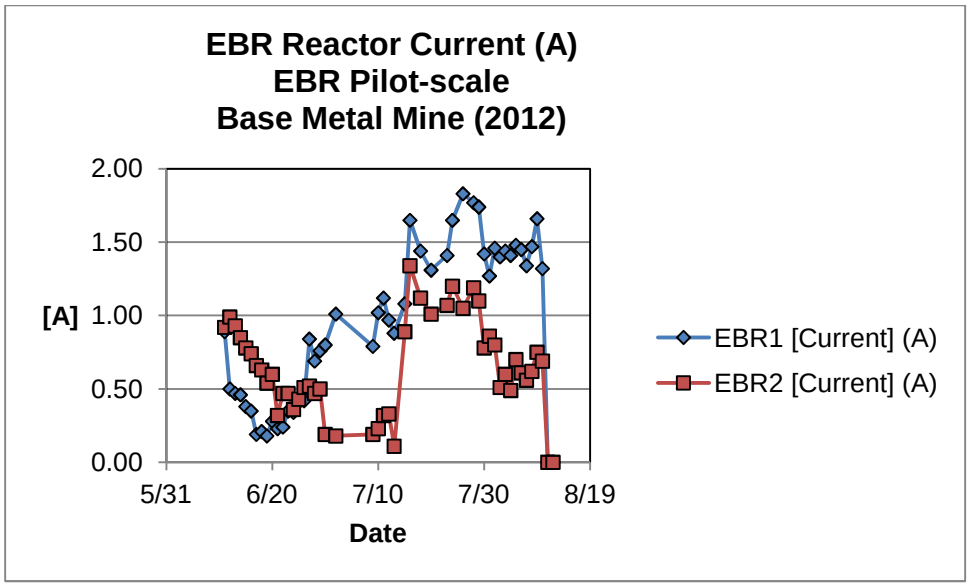

Figure D35. Reactor Current EBR Pilot-Scale (2012).

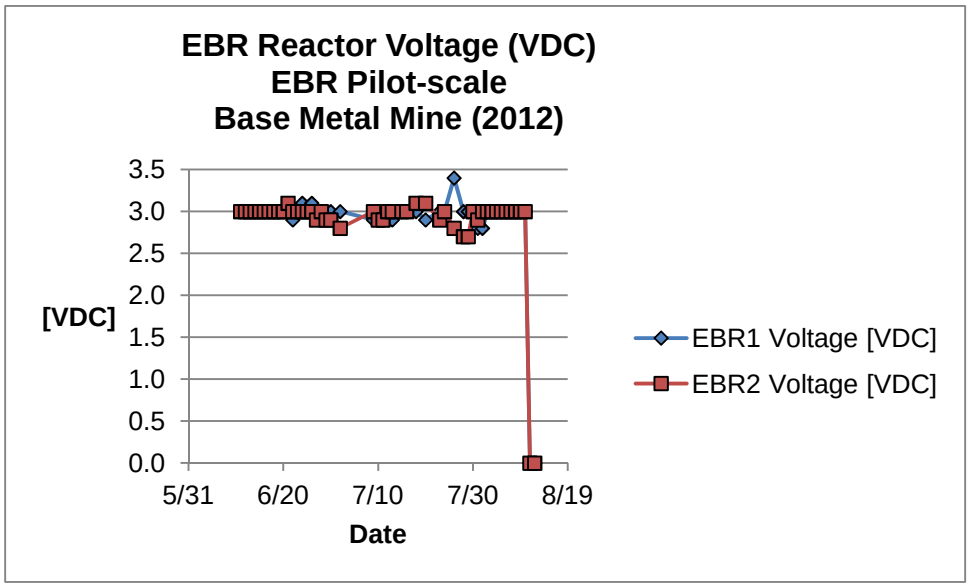

Figure D36. Reactor Voltage EBR Pilot-Scale (2012).

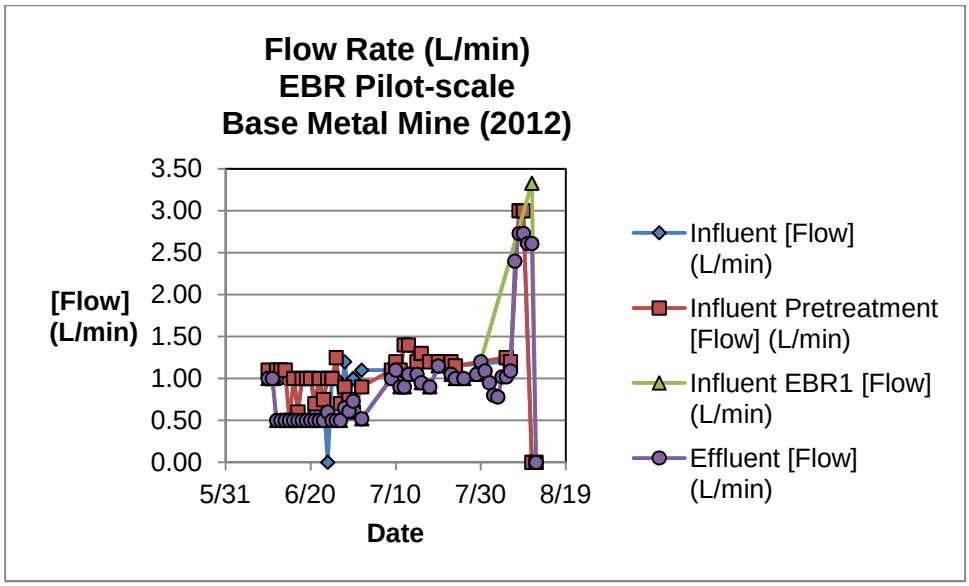

Figure D37. Flow rate EBR Pilot-Scale (2012).

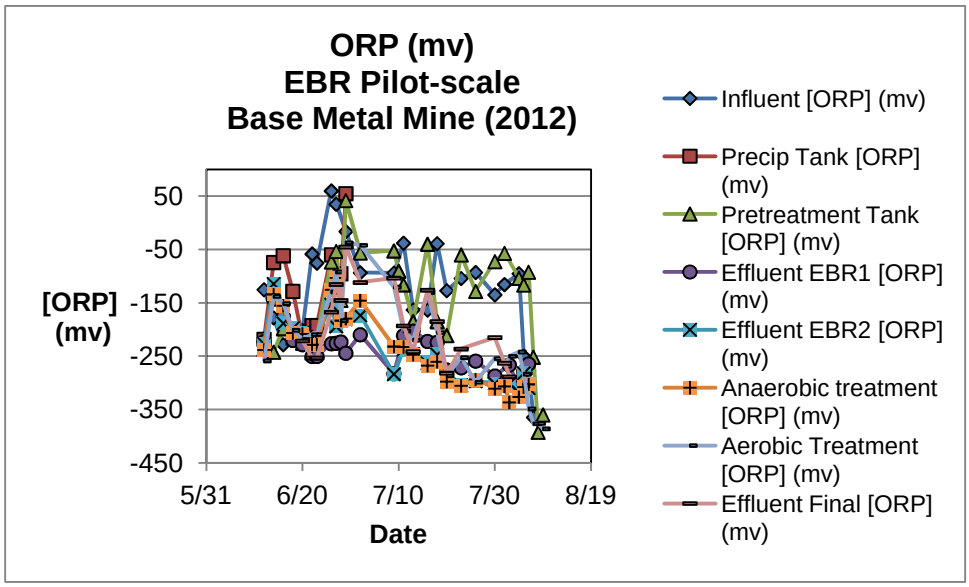

Figure D38. ORP EBR Pilot-Scale (2012).

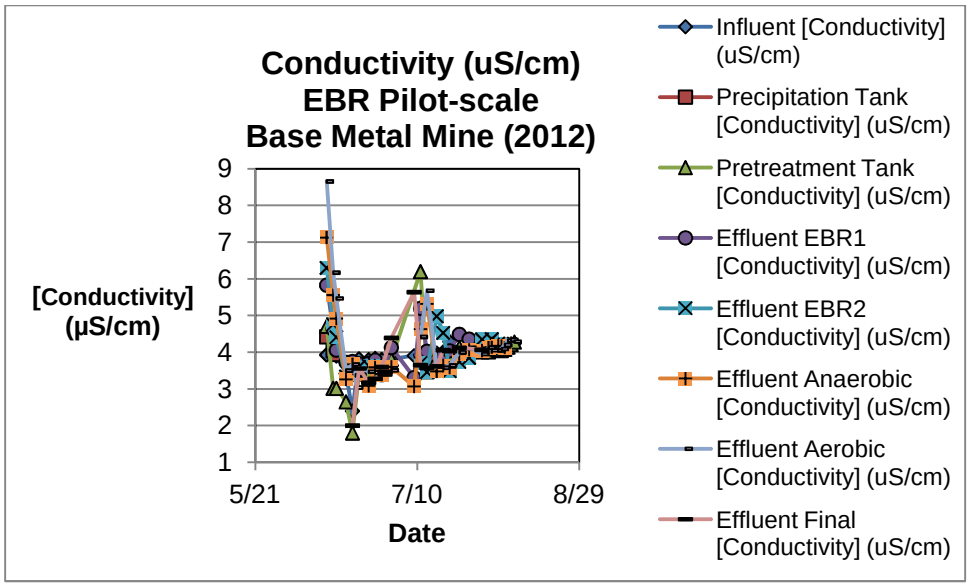

Figure D39. Conductivity EBR Pilot-Scale (2012).

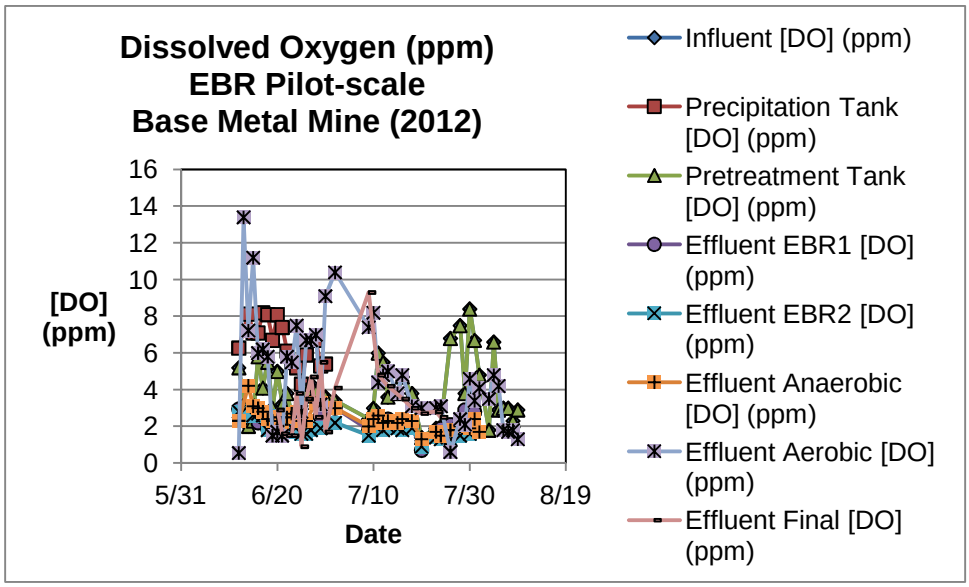

Figure D40. Dissolved Oxygen EBR Pilot-Scale (2012).

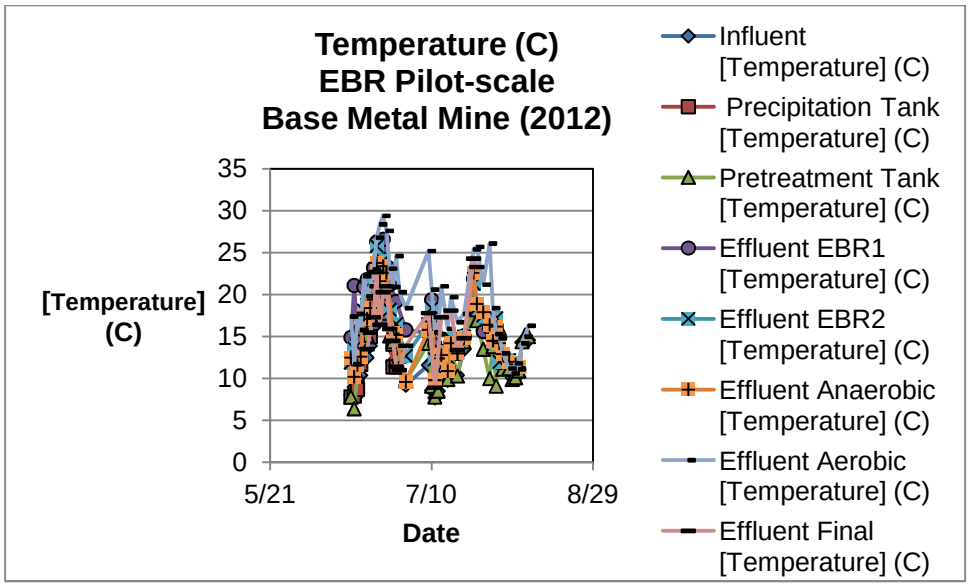

Figure D41. Temperature EBR Pilot-Scale (2012).

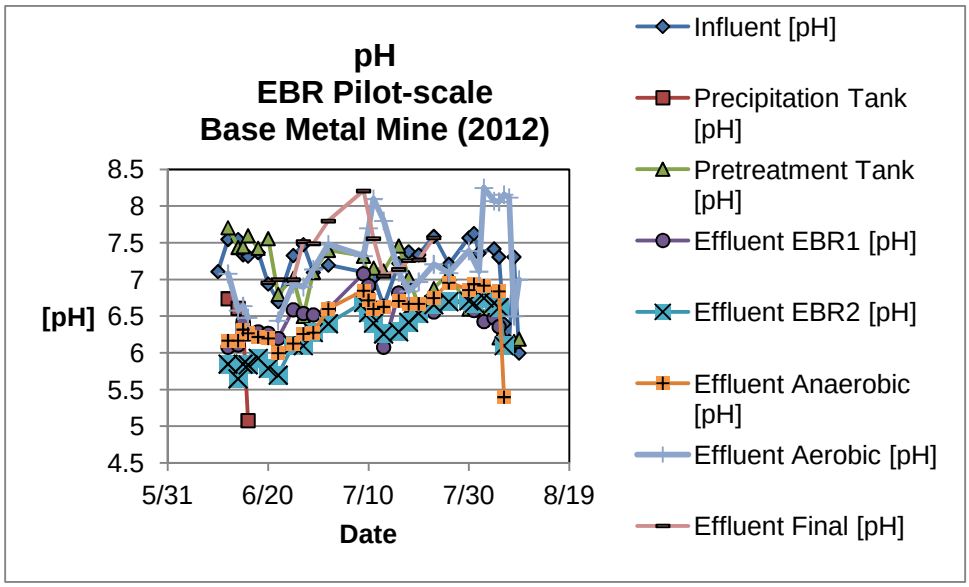

Figure D42. pH EBR Pilot-Scale (2012).

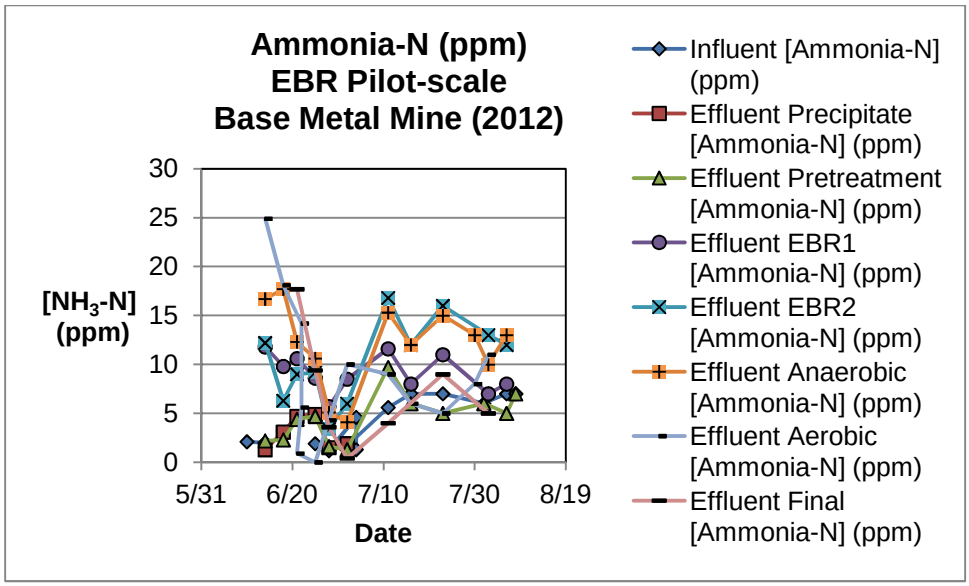

Figure D43. Ammonia EBR Pilot-Scale (2012).

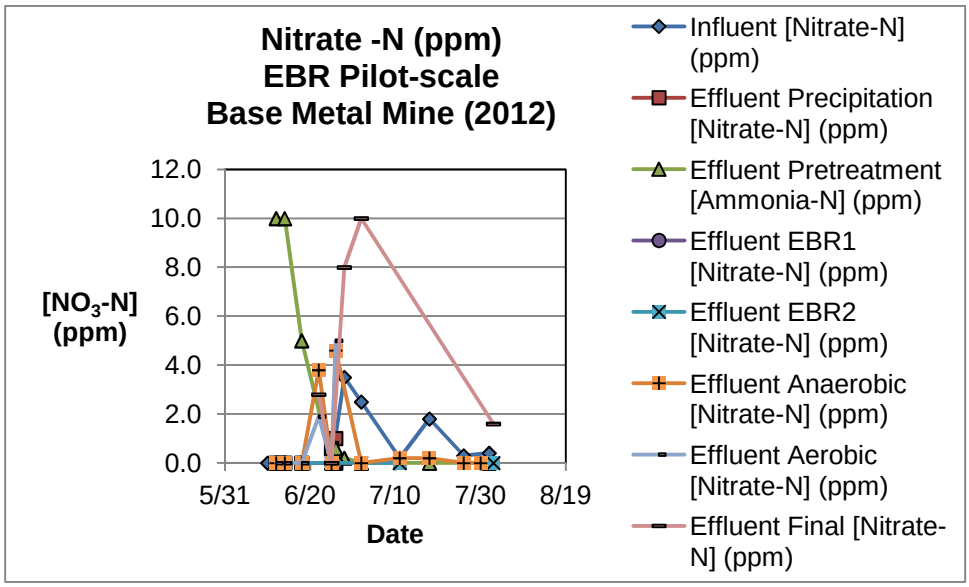

Figure D44. Nitrate EBR Pilot-Scale (2012).

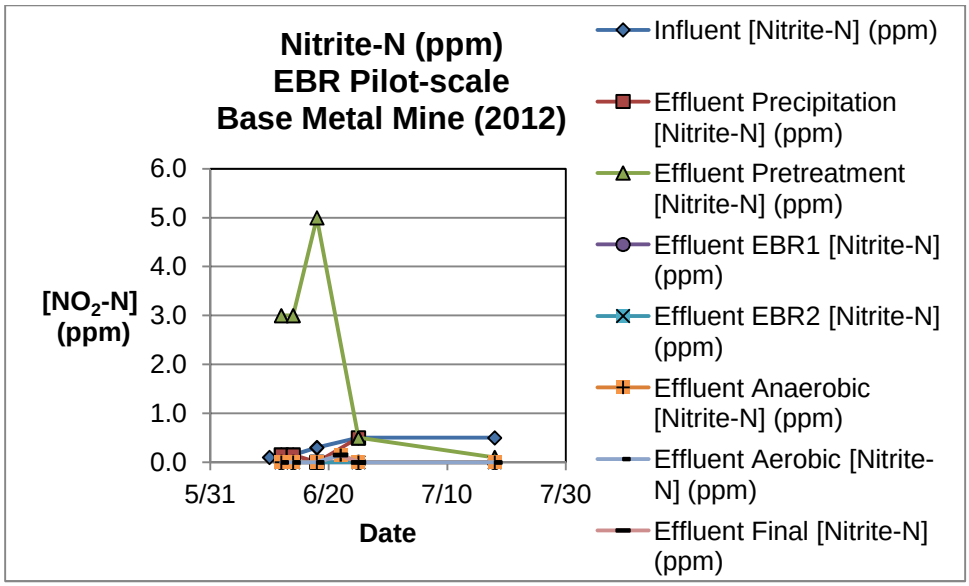

Figure D45. Nitrite EBR Pilot-Scale (2012).

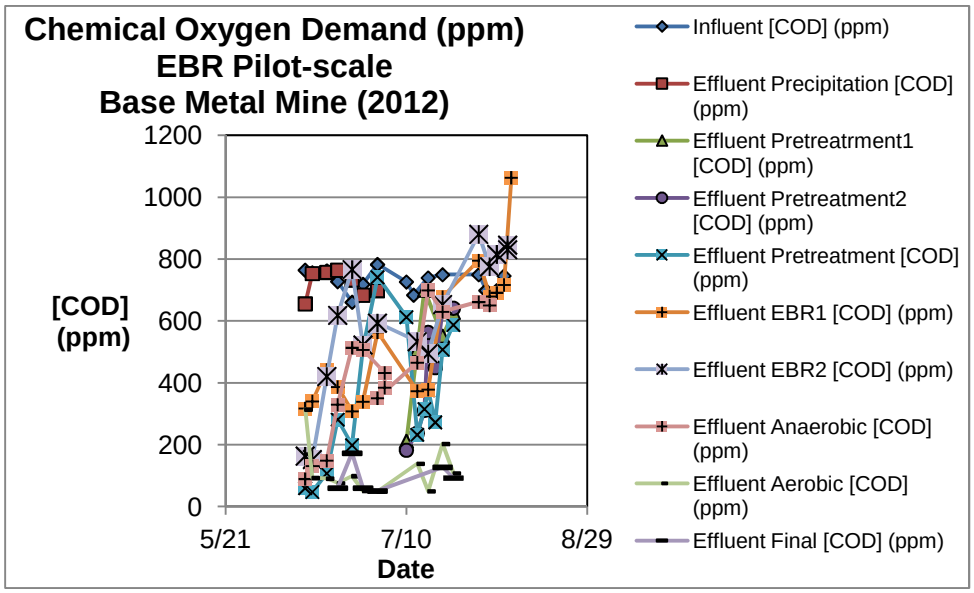

Figure D46. Chemical Oxygen Demand EBR Pilot-Scale (2012).

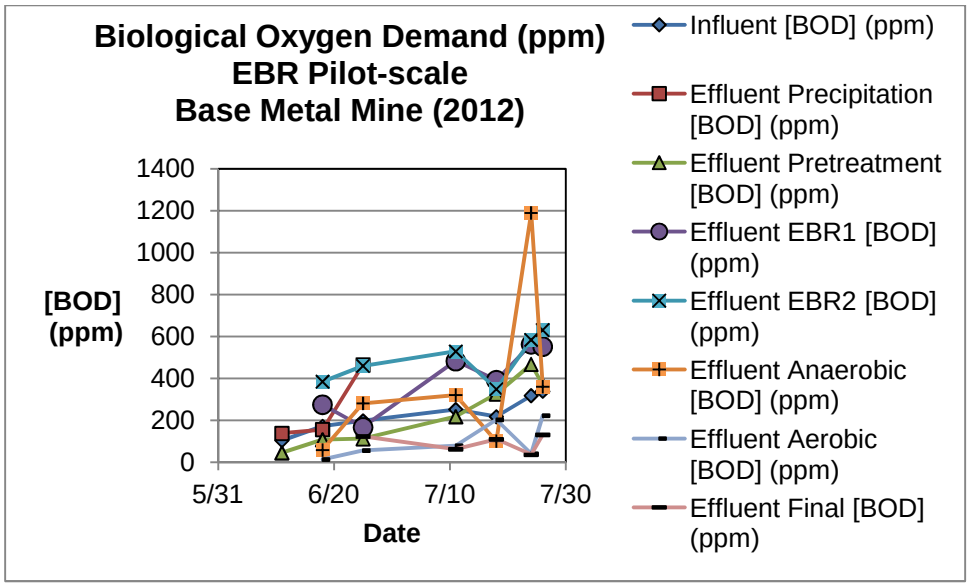

Figure D47. Biological Oxygen Demand EBR Pilot-Scale (2012).

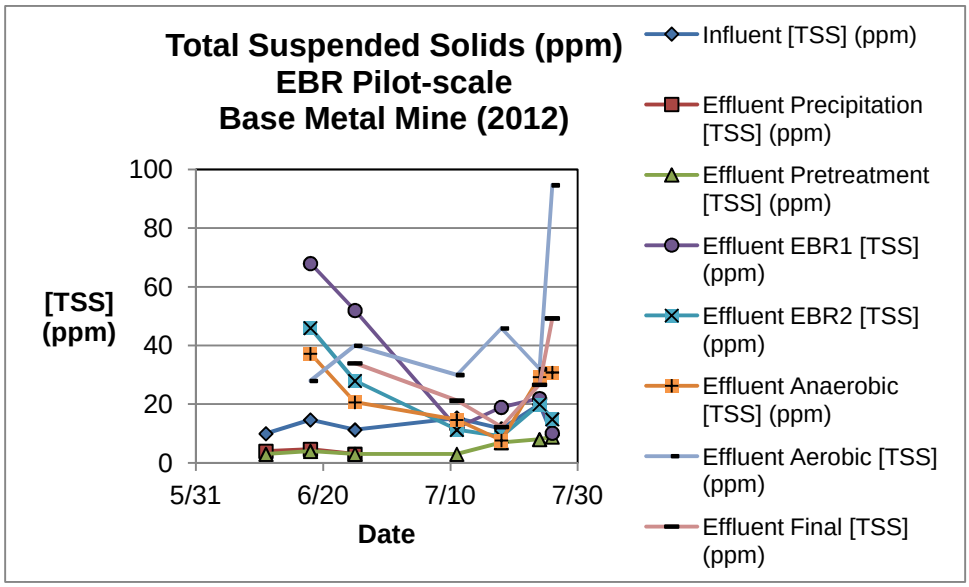

Figure D48. Total Suspended Solids EBR Pilot-Scale (2012).

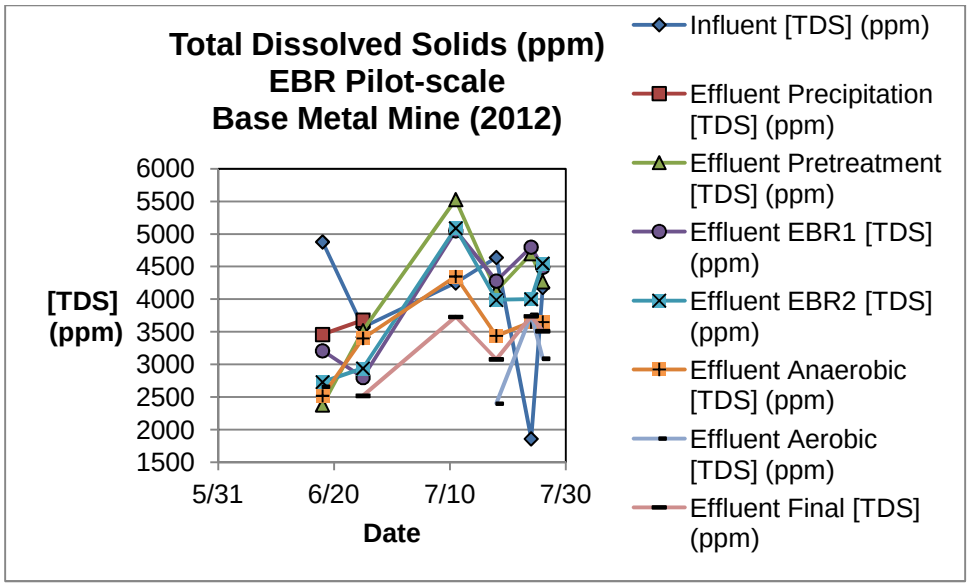

Figure D49. Total Dissolved Solids EBR Pilot-Scale (2012).

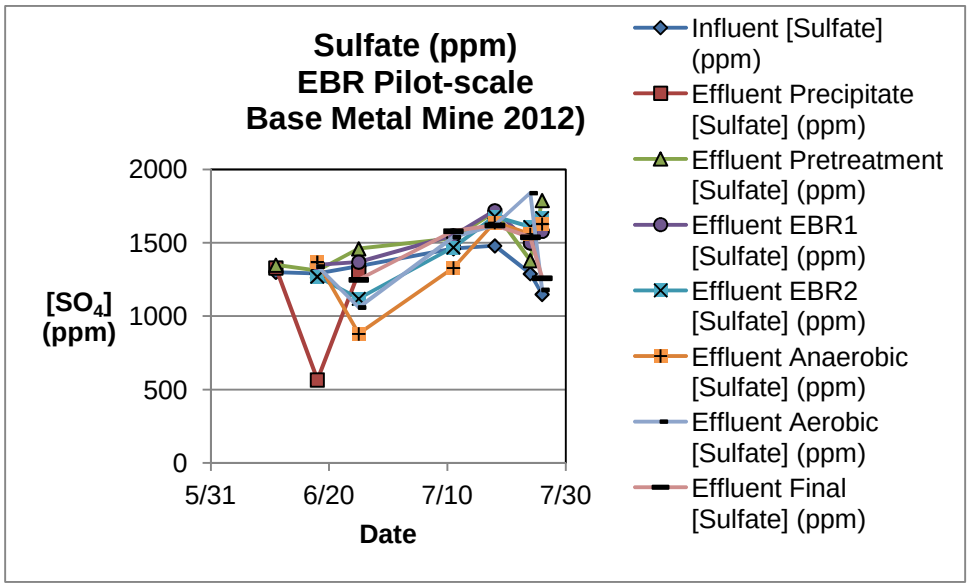

Figure D50. Sulfate EBR Pilot-Scale (2012).

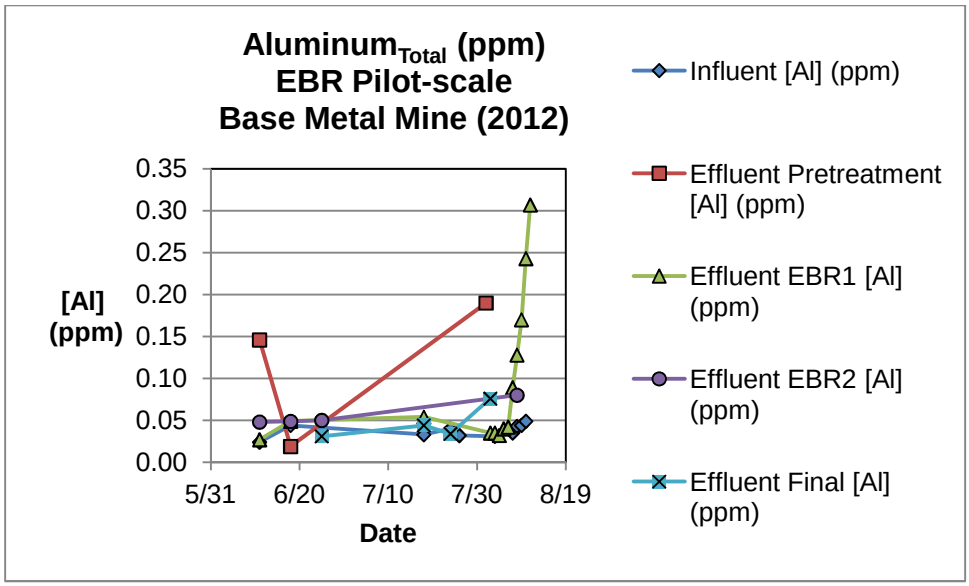

Figure D51. Aluminum$_{Total}$ EBR Pilot-Scale (2012).

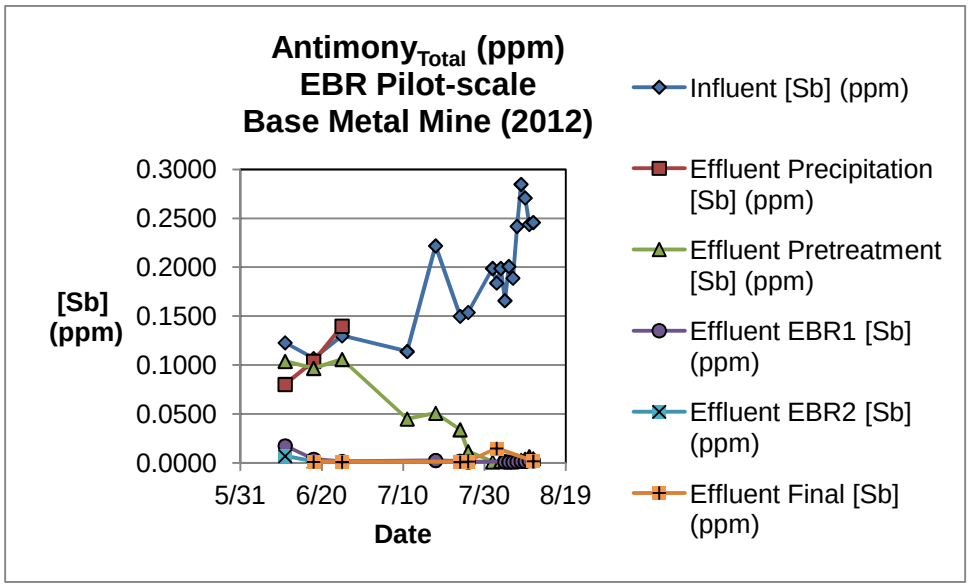

Figure D52. Antimony$_{Total}$ EBR Pilot-Scale (2012).

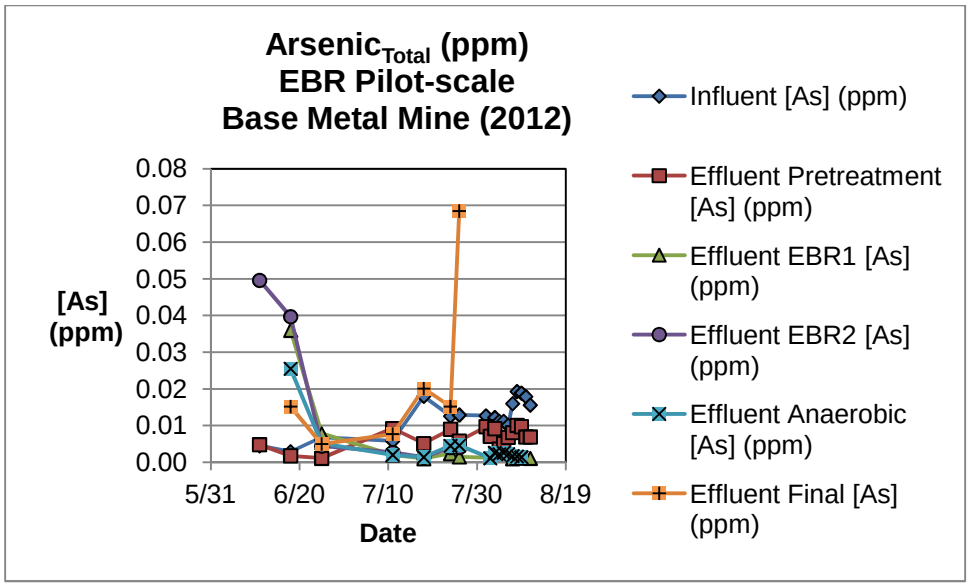

Figure D53. Arsenic$_{Total}$ EBR Pilot-Scale (2012).

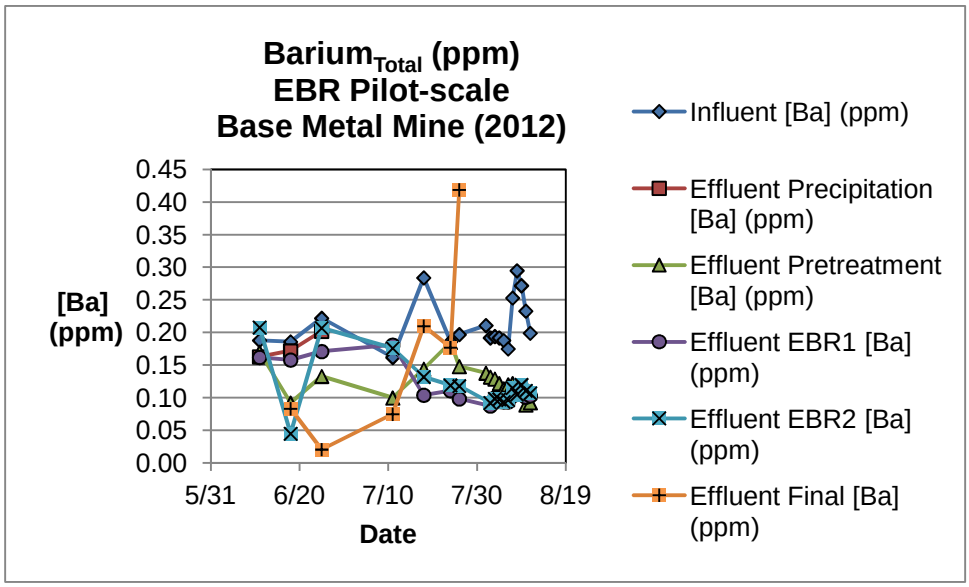

Figure D54. Barium$_{Total}$ EBR Pilot-Scale (2012).

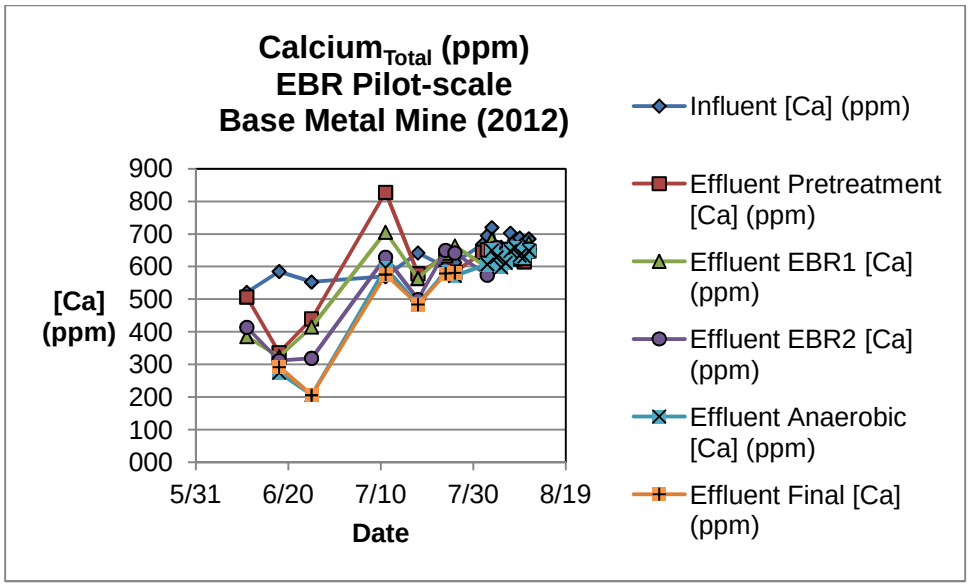

Figure D55. Calcium$_{Total}$ EBR Pilot-Scale (2012).

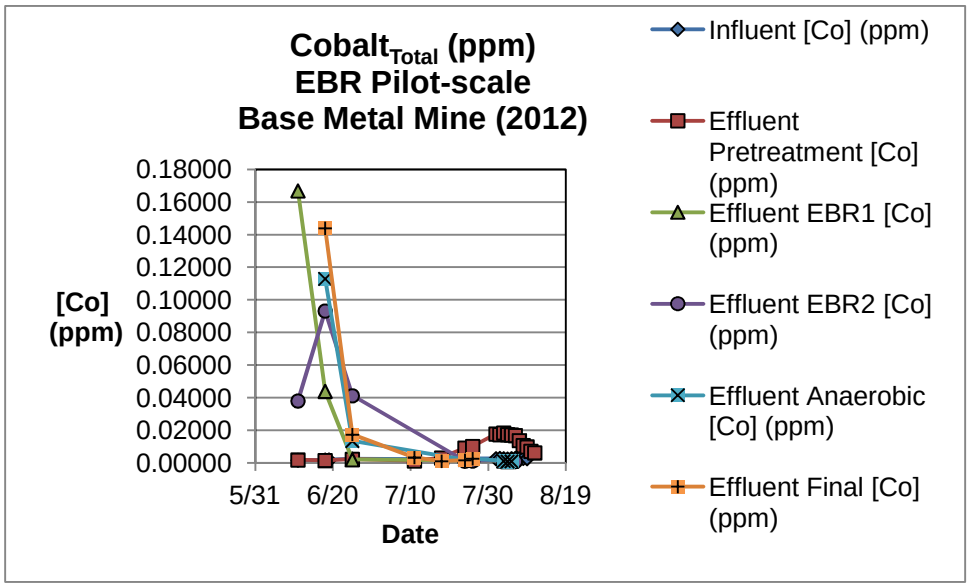

Figure D56. Cobalt$_{Total}$ EBR Pilot-Scale (2012).

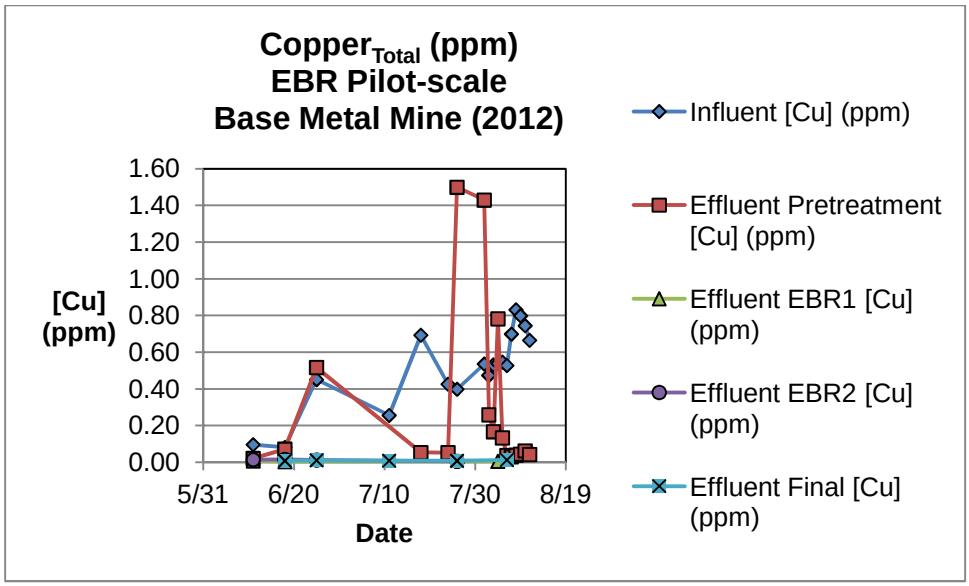

Figure D57. Copper$_{Total}$ EBR Pilot-Scale (2012).

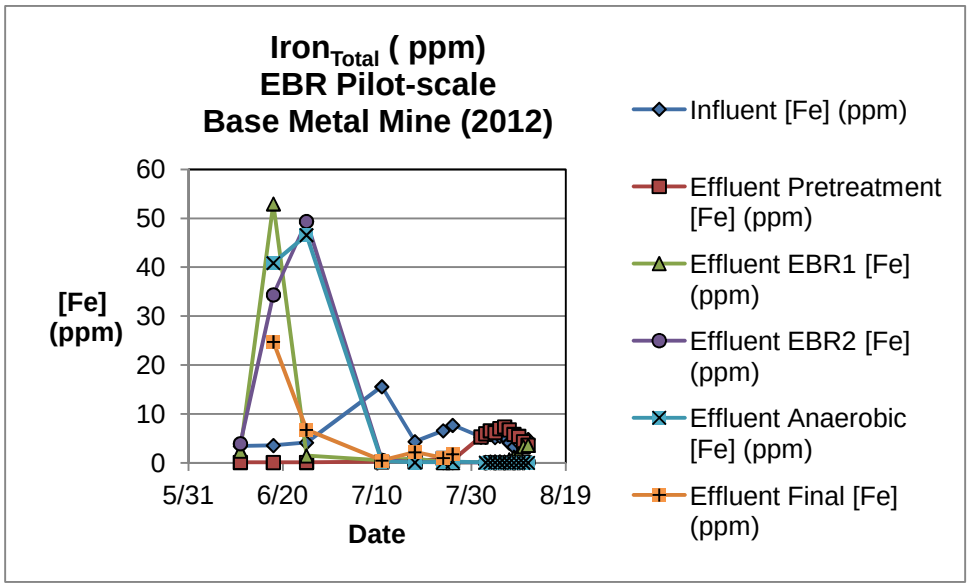

Figure D58. Iron$_{Total}$ EBR Pilot-Scale (2012).

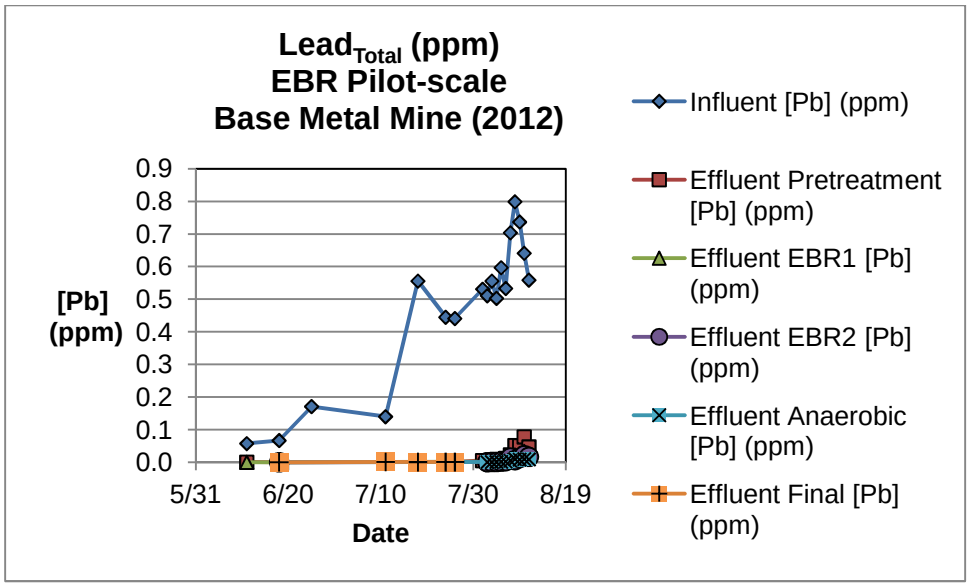

Figure D59. $Lead_{Total}$ EBR Pilot-Scale (2012).

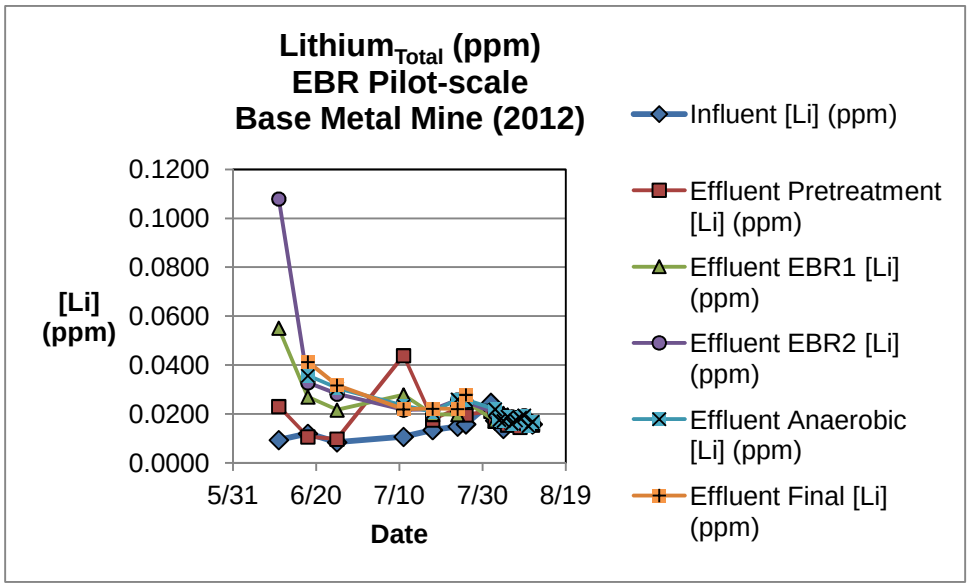

Figure D60. $Lithium_{Total}$ EBR Pilot-Scale (2012).

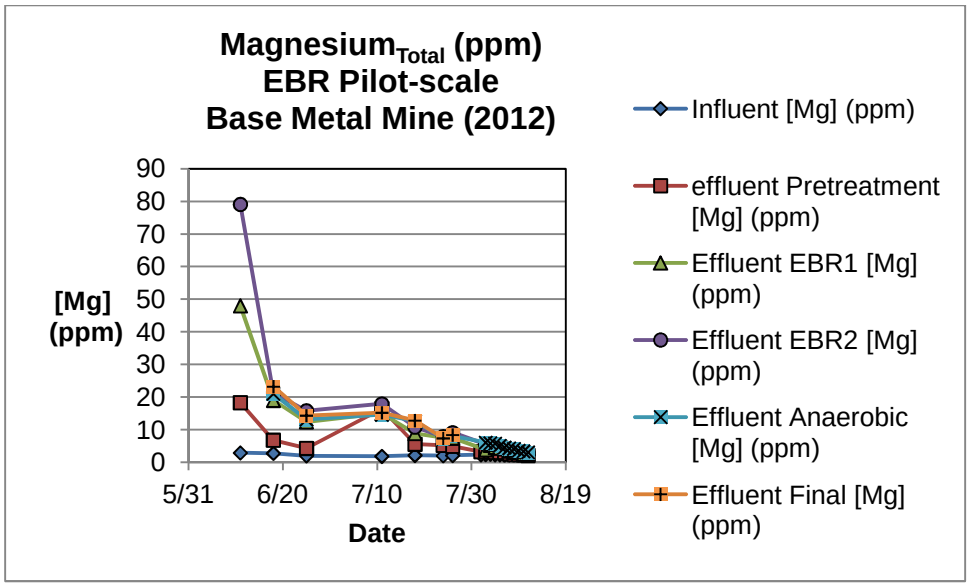

Figure D61. Magnesium$_{Total}$ EBR Pilot-Scale (2012).

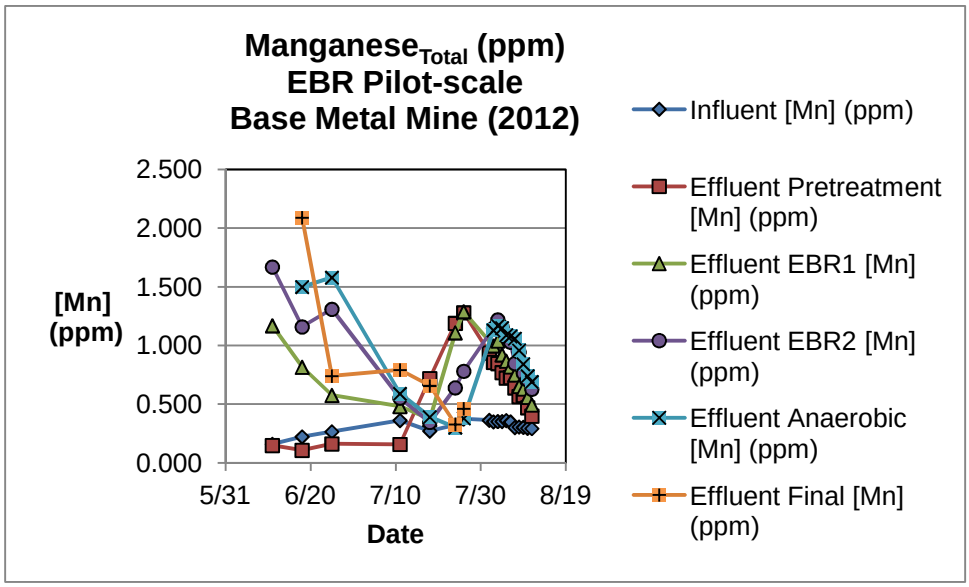

Figure D62. Manganese$_{Total}$ EBR Pilot-Scale (2012).

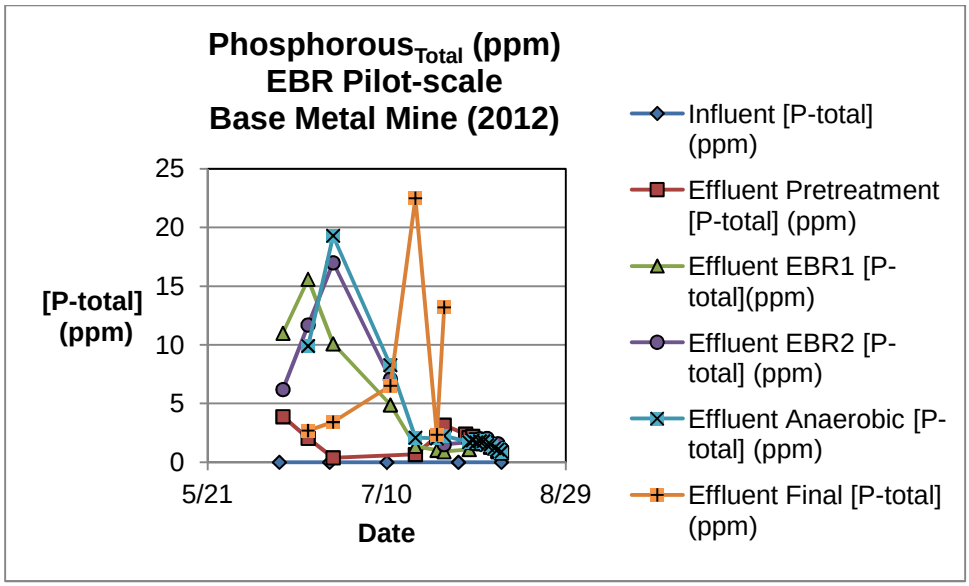

Figure D63. Phosporous$_{Total}$ EBR Pilot-Scale (2012).

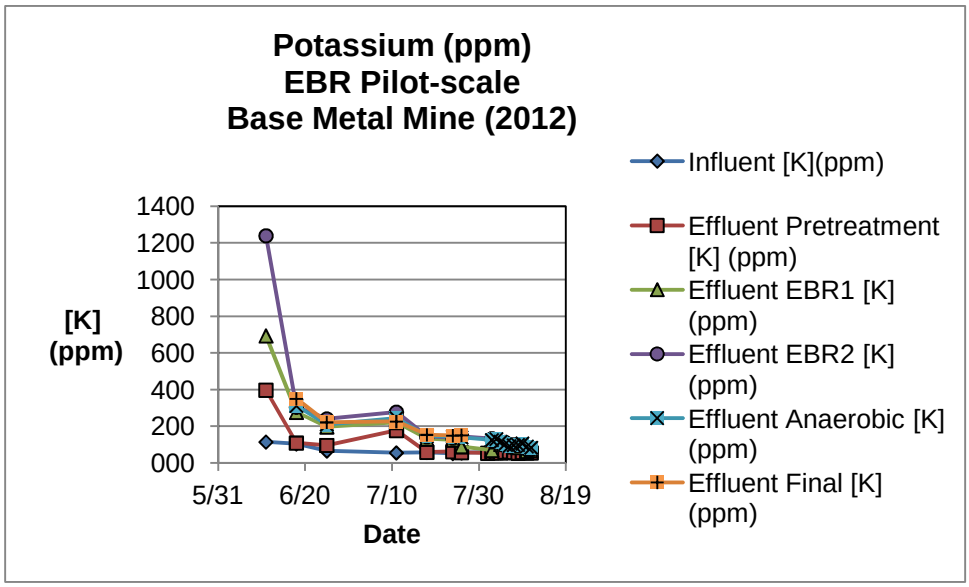

Figure D64. Potassium EBR Pilot-Scale (2012).

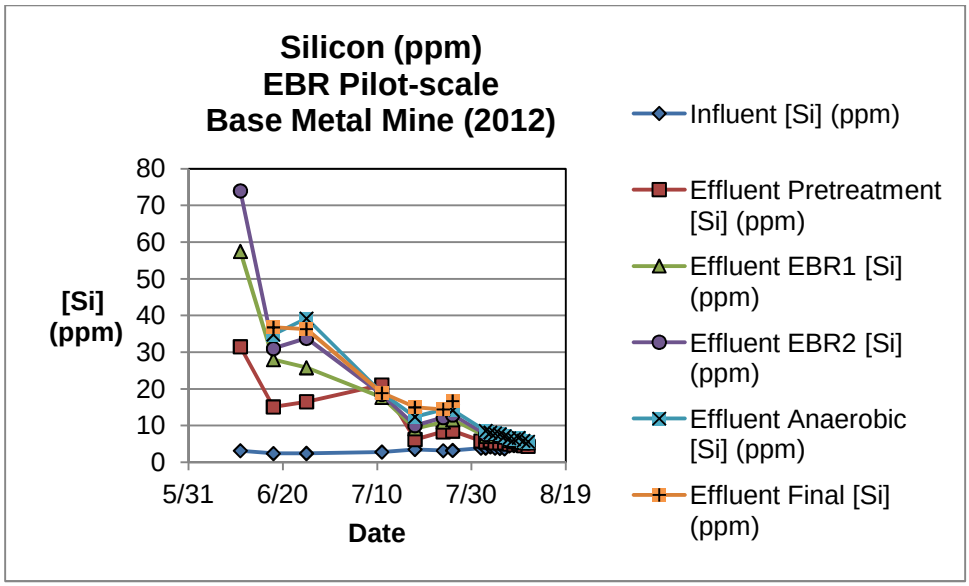

Figure D65. Silicon EBR Pilot-Scale (2012).

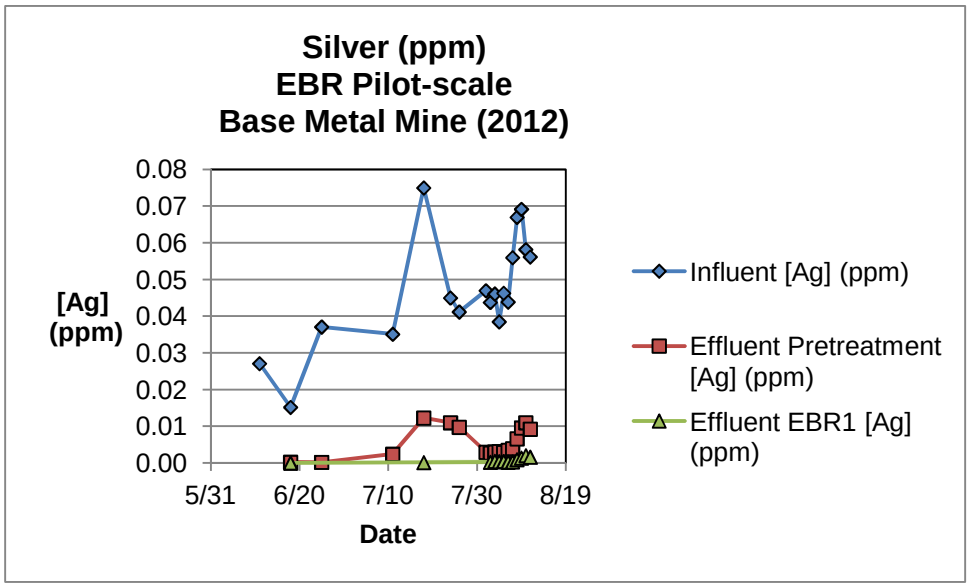

Figure D66. Silver EBR Pilot-Scale (2012).

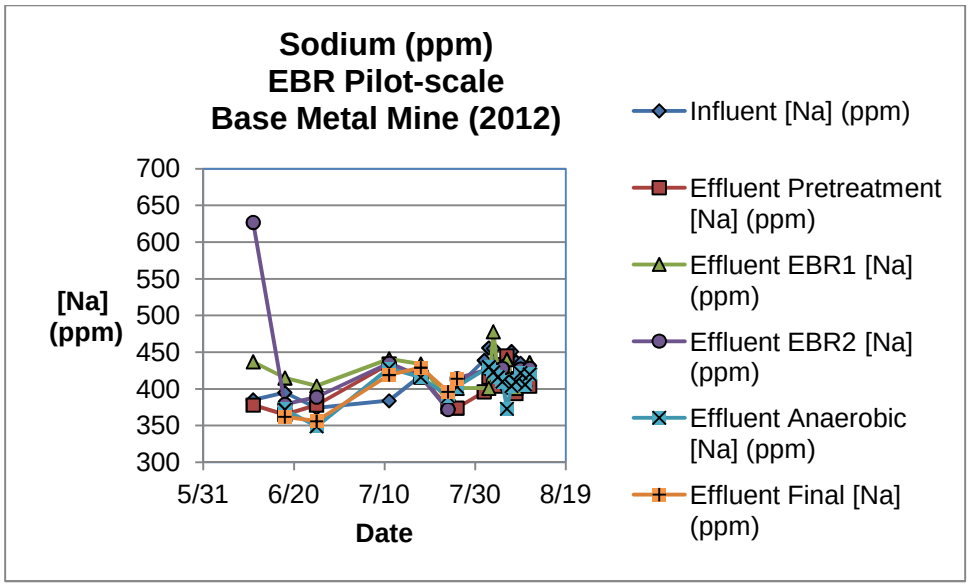

Figure D67. Sodium EBR Pilot-Scale (2012).

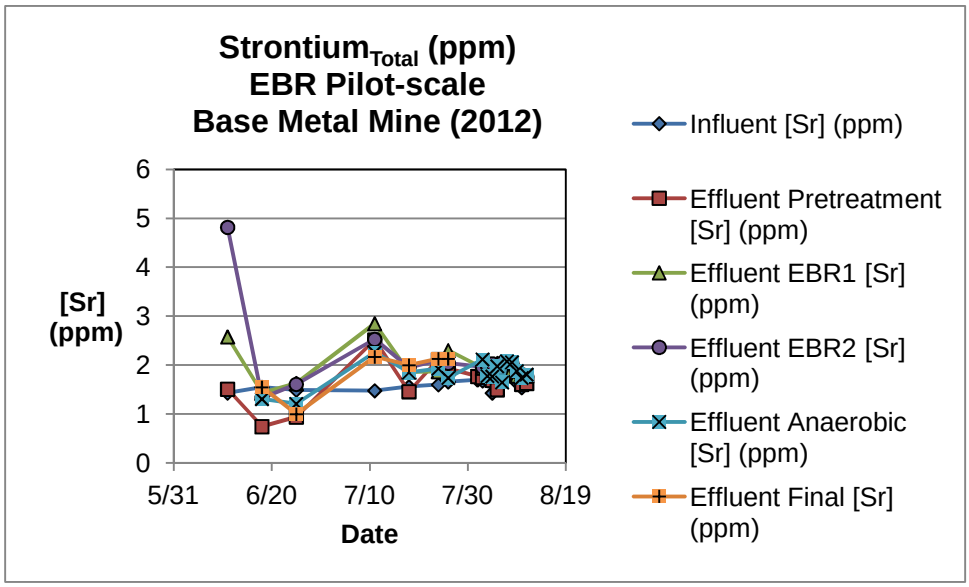

Figure D68. Strontium$_{Total}$ EBR Pilot-Scale (2012).

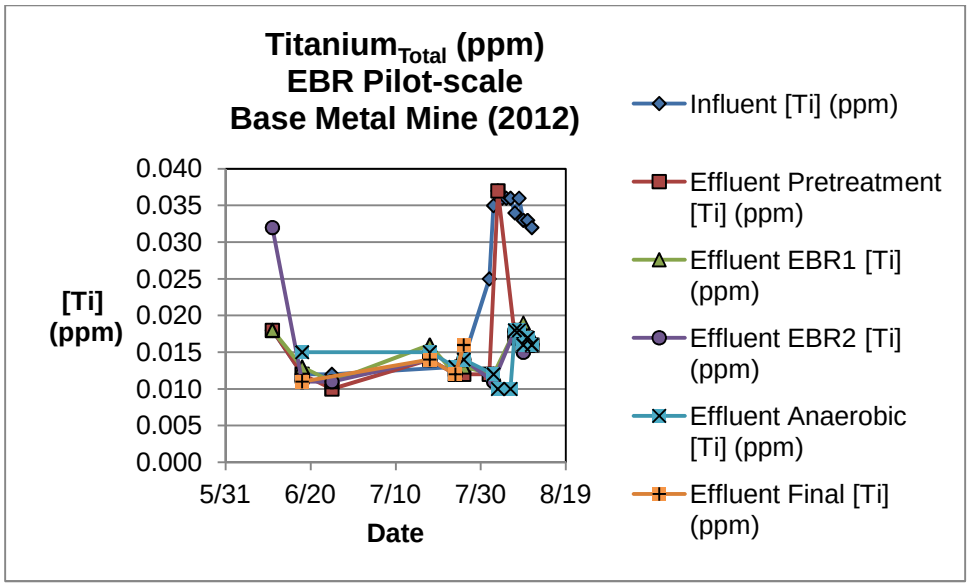

Figure D69. Titanium$_{Total}$ EBR Pilot-Scale (2012).

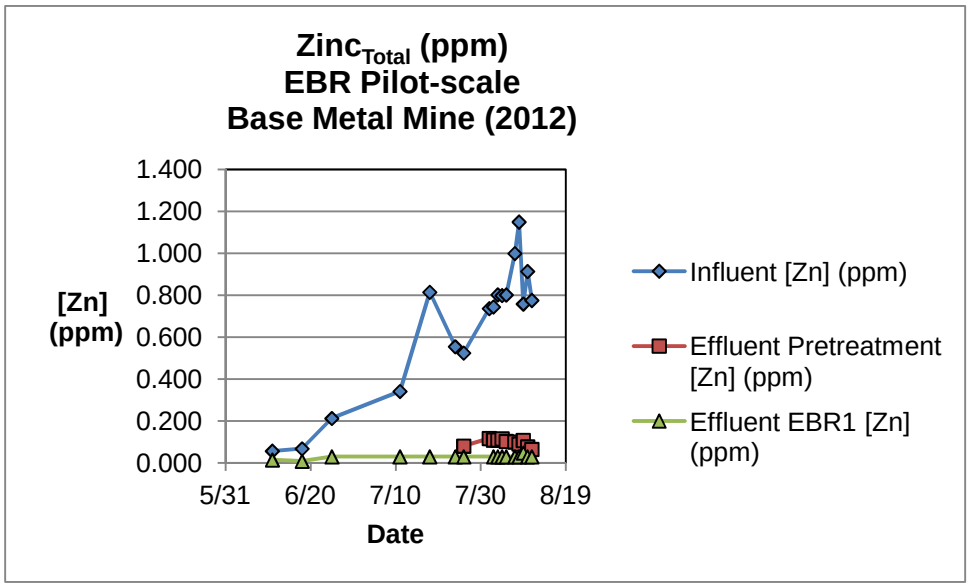

Figure D70. Zinc$_{Total}$ EBR Pilot-Scale (2012).

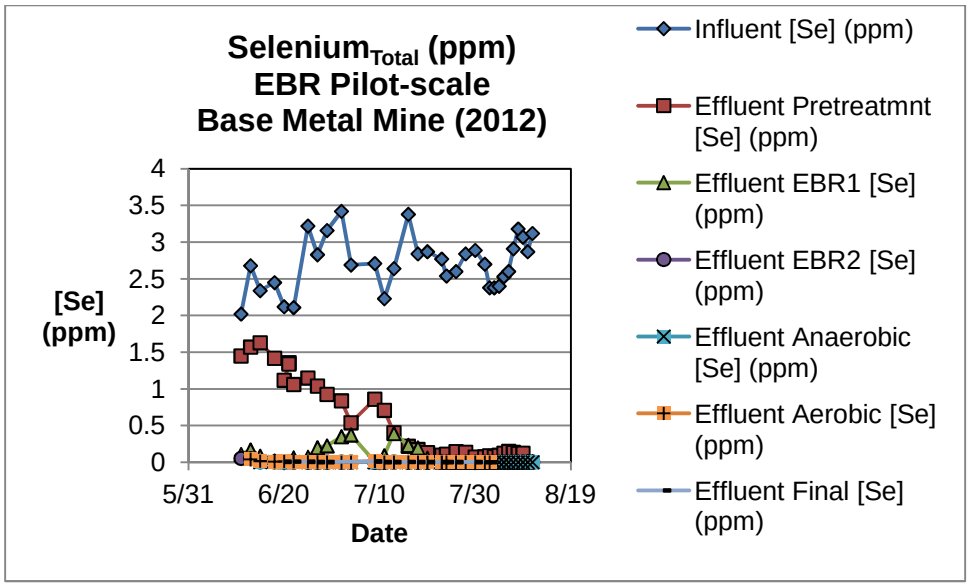

Figure D71. Selenium$_{Total}$ EBR Pilot-Scale (2012).

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